



01/17/00

Docket No. 2026-4149US4
Express Mail Label No. EJ604726585US**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE****UTILITY APPLICATION AND FEE TRANSMITTAL (1.53(b))**ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

jc688 U.S. PTO

09/483831



01/17/00

Sir:

Transmitted herewith for filing is the patent application of

First named Inventor

or Application Identifier: Stracke, M. et al.

For:

AUTOTAXIN: MOTILITY STIMULATING PROTEIN
USEFUL IN CANCER DIAGNOSIS AND THERAPY

Enclosed are

☒ 47 page(s) of specification, 1 page(s) of Abstract, 3 page(s) of claims (as originally filed in the parent application)☒ 19 sheets of drawing (Figs and) ☐ formal ☒ informal (as originally filed in the parent application)☒ 4 page(s) of Declaration and Power of Attorney☐ Unsigned☐ Newly Executed☒ Copy from prior application☐ Deletion of inventors including Signed Statement under 37 C.F.R. § 1.63(d)(2)☐ page(s) of Revocation of Power of Attorney and New Power of Attorney☒ Please direct all correspondence and all telephone calls in this matter to:William S. Feiler (Reg. 26,728)
Morgan & Finnegan, LLP
345 Park Avenue
New York, NY 10154
(212) 758-4800☒ Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the combined declaration and power of attorney is supplied herein, is considered as being part of the disclosure of the accompanying application and is incorporated herein by reference.☐ Microfiche Computer Program (Appendix)

002710 1682460

- 002110 FEB 24 09 48 33
- ☒ 46 page(s) of Sequence Listing
- ☒ computer readable disk containing Sequence Listing
- ☒ Statement under 37 C.F.R. § 1.821(f) that computer and paper copies of the Sequence Listing are the same
- ☐ Certified copy of Priority Document(s)
- ☐ English translation documents
- ☒ Information Disclosure Statement
- ☐ Copy of ___ cited references
- ☒ Preliminary Amendment
- ☒ Return receipt postcard (MPEP 503)
- ☒ Assignment Papers (assignment cover sheet and assignment documents)
- ☐ A check in the amount of \$40.00 for recording the Assignment.
- ☒ Assignment papers as filed in application Serial No. 08/346,455, recorded on Reel 7324, Frame 0799 on February 10, 1995.
- ☐ Certification of chain of title pursuant to 37 C.F.R. § 3.73(b).
- ☒ This is a ☒ continuation ☐ divisional ☐ continuation-in-part (C-I-P) of prior application serial no. 08/977,221, herein incorporated by reference.
- ☒ Cancel in this application original claims 1-19 of the parent application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
- ☒ A Preliminary Amendment is enclosed. (Claims added by this Amendment have been properly numbered consecutively beginning with the number following the highest numbered original claim in the prior application.
- ☒ The status of the parent application is as follows:
- ☐ A Petition For Extension of Time and a Fee therefor has been or is being filed in the parent application to extend the term for action in the parent application until _____.
- ☐ A copy of the Petition for Extension of Time in the co-pending parent application is attached.
- ☒ No Petition For Extension of Time and Fee therefor are necessary in the co-pending parent application.
- ☐ Please abandon the parent application at a time while the parent application is pending or at a time when the petition for extension of time in that application is granted and while this application is pending has been granted a filing date, so as to make this application co-pending.
- ☐ Transfer the drawing(s) from the parent application to this application.

Express Mail Label No. EJ604726585US

- ☒ Amend the specification by inserting before the first line the sentence:
This is a ☒ continuation ☐ divisional ☐ continuation-in-part of co-pending application Serial
No. 08/977,221 filed November 24, 1997, herein incorporated by reference.

I. CALCULATION OF APPLICATION FEE

					Basic Fee
	Number Filed	Number Extra	Rate		\$690.00
Total					
Claims	6	-20= 0	x \$18.00		\$
Independent					
Claims	1	- 3= 0	x \$78.00		\$
Multiple Dependent Claims					
		<input checked="" type="checkbox"/> yes Additional fee	= \$260.00	\$	\$260.00
		<input type="checkbox"/> no Additional fee	= NONE		
Total:					<u>\$950.00</u>

- ☐ A statement claiming small entity status is attached or has been filed in the above-identified parent application and its benefit under 37 C.F.R. § 1.28(a) is hereby claimed. Reduced fees under 37 C.F.R. § 1.9(F) (50% of total) paid herewith \$_____.
- ☒ A check in the amount of \$ 950.00 in payment of the application filing fees is attached.
- ☒ Charge Fee(s) to Deposit Account No. 13-4500. Order No. 2026-4149US4. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.
- ☒ The Assistant Commissioner is hereby authorized to charge any additional fees which may be required for filing this application, or credit any overpayment to Deposit Account No. 13-4500 Order No. 2026-4149US4. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By: Dorothy R. Auth
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Dated: January 17, 2000

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EJ604726585US

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PATENT
Docket No. 2026-4149US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Stracke, M. et al.

Group Art Unit: To Be Assigned

Continuation Application

of Serial No. : 08/977,221

Examiner: To Be Assigned

Filed : Herewith

For : AUTOTAXIN: MOTILITY STIMULATING PROTEIN
USEFUL IN CANCER DIAGNOSIS AND THERAPY

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EXPRESS MAIL CERTIFICATE

Express Mail Label No. EJ604726585US

Date of Deposit January 17, 2000

I hereby certify that the following attached paper(s) or fee

1. Utility Application and Fee Transmittal (1.53(b));
2. Copy of Patent Application (including 47 pages of specification; 3 pages of claims, 1 page of abstract and 19 pages of drawings);
3. Copy of Combined Declaration and Power of Attorney;
4. Copy of Associate Power of Attorney;
5. Copy of Assignment;
6. Paper Copy of Sequence Listing (46 pages);
7. 1 computer disk containing Sequence Listing;
8. Statement Under 37 C.F.R. §1.821(f);
9. Copy of PTO-1449 (9 sheets) and ;
10. Return Postcard.

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, BOX PATENT APPLICATION, Washington, D.C. 20231.

Francisco J. Garcia

(Typed or printed name of person mailing paper(s) or fee)

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EJ604726585US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Stracke, M. et al.

Group Art unit : 1652

Continuation Application
of Serial No. : 08/977,221

Examiner : Longton, E.

Filed : Herewith

For : AUTOTAXIN: MOTILITY STIMULATING PROTEIN
USEFUL IN CANCER DIAGNOSIS AND THERAPY

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

Sir:

Prior to examination and calculation of the filing fee, please amend the
application as follows.

IN THE CLAIMS

Please cancel claims 1-19 and add the following new claims:

20. An isolated polypeptide comprising an amino acid sequence of human
autotaxin, wherein said polypeptide thereof has cell motility activity.

21. The polypeptide of claim 20, wherein said polypeptide is a fragment
thereof having at least 5 amino acids.

22. The polypeptide of claim 20 or 21, wherein said polypeptide is bound to a solid support.

23. A method of purifying the autotaxin polypeptide of claim 20 or 21, comprising the steps of:

- i) collecting and concentrating supernatant from cultured A2058 human melanoma cells whereby a first preparation of said polypeptide is produced;
- ii) salt fractionating said first preparation to produce a second polypeptide preparation;
- iii) isolating said polypeptide from said second preparation so that said polypeptide is obtained in substantially pure form.

24. The method of claim 23, wherein said isolating step is effected by column chromatography.

25. A recombinant autotaxin polypeptide according to claim 20 or 21.

REMARKS

Applicants respectfully request favorable consideration of the present application and claims. Early and favorable action by the Examiner is earnestly solicited.

No additional fee is believed to be necessary.

The Commissioner is hereby authorized to charge any additional fees which may be required for this amendment, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2026-4149US4.

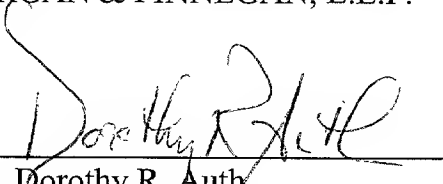
In the event that an extension of time is required, or which may be required in addition to that requested in a petition and for an extension of time, the Commissioner is

requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. 13-4500, Order No. 2026-4149US4. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By:


Dorothy R. Auth
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Dated: January 17, 2000

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AUTOTAXIN: MOTILITY STIMULATING PROTEIN USEFUL
IN CANCER DIAGNOSIS AND THERAPY

This application is a continuation-in-part of application serial no. 08/249,182 filed May 25, 1994, which is a continuation-in-part of application serial no. 07/822,043 filed on Jan. 17, 1992.

Field of the Invention

The present invention relates, in general, to a motility stimulating and compositions comprising the same. In particular, the present invention relates to a purified form of the protein and peptides thereof, for example, autotaxin (herein alternative referred to as "ATX"); a DNA segment encoding autotaxin; recombinant DNA molecules containing the DNA segment; cells containing the recombinant DNA molecule; a method of producing autotaxin; antibodies to autotaxin; and methods of cancer diagnosis and therapy using the above referenced protein or peptides thereof and DNA segments.

Background of the Invention

Cell motility plays an important role in embryonic events, adult tissue remodeling, wound healing, angiogenesis, immune defense, and metastasis of tumor cells (Singer, 1986). In normal physiologic processes, motility is tightly regulated. On the other hand, tumor cell motility may be aberrantly regulated or autoregulated. Tumor cells can respond in a motile fashion to a variety of agents. These include host-derived factors such as scatter factor (Rosen, et al., 1989) and growth factors (Kahan, et al., 1987; Stracke, et al.; Tamm, et al., 1989; Wang, et al. 1990; and Jouanneau, et al. 1991), components of the extracellular matrix (McCarthy, et al. 1984), and tumor-secreted or autocrine factors (Liotta, et al. 1988; Ruff, et al. 1985; Atnip, et al. 1987; Ohnishi, et al. 1990; Silletti, et al. 1991; and Watanabe, et al. 1991).

Many types of host-derived soluble factors act in a paracrine fashion to stimulate cell locomotion.

Motility-stimulating proteins called "scatter factors" have been identified which are produced by embryonic fibroblasts and by smooth muscle cells (Stoker, et al. 1987). Scatter factors stimulate random and directed motility by epithelial cells, keratinocytes, vascular endothelial cells and carcinoma cells (Stoker, et al. 1987; Rosen, et al. 1990; and Weidner, et al. 1990), but not fibroblasts. In addition, a number of host-secreted growth factors have been demonstrated to stimulate motility in tumor cells, including nerve growth factor (Kahan, et al. 1987) insulin-like growth factor-I (Stracke, et al. 1988), interleukin-6 (Tamm, et al. 1989), interleukin-8 (Wang, et al. 1990), and acidic fibroblast growth factor (Jouanneau, et al. 1991). These paracrine factors may influence "homing" or the directionality of tumor cell motility.

In contrast to these host-derived factors, many types of tumor cells have been found to produce proteins termed "autocrine motility factors" which stimulate motility by the same tumor cells which make the factor (Liotta, et al. 1986). Autocrine motility factors are not specific for a given type of cancer cell but have a wide spectrum of activity on many types of cancer cells (Kohn, et al. 1990), with little effect on normal fibroblasts or leukocytes.

Autocrine motility factors identified to date act through cell-surface receptors (Stracke, et al. 1987; Nabi, et al. 1990; Watanabe, et al. 1991) resulting in pseudopodial protrusion (Guirguis, et al. 1987) leading to both random and directed migration (Liotta, et al. 1986; Atnip, et al. 1987; Ohnishi, et al. 1990).

Prior studies of human A2058 melanoma cells have demonstrated that these cells are a particularly rich source of autocrine motility factors. An autocrine motility factor with a molecular mass of approximately 60 kDa has been previously isolated from the conditioned

media of these cells. (Liotta, et al. 1986). Similar tumor cells derived or induced factors with the same molecular weight have subsequently been reported and purified by several investigators (Atnip, et al. 1987; Schnor, et al. 1988; Ohnishi, et al. 1990; Silletti, et al. 1991; Watanabe et al. 1990). Such factors are thought to play a key role in tumor cell invasion.

Most of the motility factors identified to date have not been purified to homogeneity and have not been sequenced. The novel tumor motility factor of the present invention, named herein as autotaxin ("ATX"), has been purified and verified to be a homogeneous sample by two-dimensional gel electrophoresis. The protein of the present invention is unique from any previously identified or purified motility factor. The molecular size of ATX is about 125 kilo Daltons ("kDa") and it has an isoelectric point of approximately 7.7. ATX stimulates both random and directed migration of human A2058 melanoma cells at picomolar concentrations. The activity of the ATX factor is completely sensitive to inhibition by pertussis toxin. No significant homology has been found to exist between the protein of the invention and any mammalian protein including previous factors known to stimulate cell motility.

There is a great clinical need to predict the aggressiveness of a patient's individual tumor, to predict the local recurrence of treated tumors and to identify patients at high risk for development of invasive tumors. The present invention provides a functional marker which is functionally related to the invasive potential of human cancer. The invention further provides an assay for this secreted marker in body fluids, or in tissues. The assay of the invention can be used in the detection, diagnosis, and treatment of human malignancies and other inflammatory, fibrotic, infectious or healing disorders.

SUMMARY OF THE INVENTION

The present invention relates, generally, to a motility stimulating protein and corresponding peptides thereof, and to a DNA segment encoding same. A human cDNA clone encoding a tumor cell motility-stimulating protein, herein referred to as autotaxin or "ATX", reveals that this protein is an ecto/exoenzyme with significant homology to the plasma cell membrane differentiation antigen PC-1. ATX is a 125 kDa glycoprotein, previously isolated from a human melanoma cell line (A2058), which elicits chemotactic and chemokinetic responses at picomolar to nanomolar concentrations.

It is a specific object of the present invention to provide autotaxin and peptide fragments thereof.

It is a further object of the present invention to provide a DNA segment that encodes autotaxin and a recombinant DNA molecule comprising same. It is a further object of the present invention to provide a cell that contains such a recombinant molecule and a method of producing autotaxin using that cell.

Another object of the present invention is the identification of a transmembrane domain of the human liver autotaxin protein and its apparent absence in tumorous forms of autotaxin. The tumorous form of autotaxin appears to be a secreted protein. The present invention relates to utilization of the different sites of localization for diagnosis and prognosis of the stages of tumor progression. Further, the invention relates to treatment methods, designed to advantageously block the secreted form of autotaxin activity while having little effect on the membrane-bound form of autotaxin.

Yet another object of the present invention relates to the identification of a highly variable region within the autotaxin gene, called a "hot spot". The variations in sequence apparently result in mutations, insertions, deletions and premature termination of

translation. The present invention relates to manipulating this region so as to alter the activity of the protein. Further, the hot spot can serve as a marker in tumor diagnosis differentiating between different forms of the autotaxin protein.

It is yet another object of the present invention to provide a method of purifying autotaxin.

It is a further object of the present invention to provide cloned DNA segments encoding autotaxin and fragments thereof. The cDNA encoding the entire autotaxin protein contains 3251 base pairs, and has an mRNA size of approximately 3.3 kb. The full-length deduced amino acid sequence of autotaxin comprises a protein of 915 amino acids. Database analysis of the ATX sequence revealed a 45% amino acid identity (including 30 out of 33 cysteines) with PC-1, a pyrophosphatase/type I phosphodiesterase expressed on the surface of activated B cells and plasma cells. ATX, like PC-1, was found to hydrolyze the type I phosphodiesterase substrate p-nitrophenyl thymidine-5'-monophosphate. Autotaxin now defines a novel motility-regulating function for this class of ecto/exo-enzymes.

Further objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Fractionation of ATX by hydrophobic interaction. A 200 ml sample of A2058 conditioned media was chromatographed on a 200 mL column of phenyl Sepharose-4B. Buffer A was 50 mM Tris (pH 7.5), 5% methanol, and 1.2 M ammonium sulfate. Buffer B was 50 mM Tris (pH 7.5), 5% methanol and 50% ethylene glycol. The gradient (----) represents a double linear gradient with decreasing ammonium sulfate (1.2 to 0.0 M) and increasing ethylene glycol (0 to 50%). Absorbance was monitored at 280 nm (——) and indicated that most of the protein did not bind to the column. Ten ml fractions were assayed for

motility stimulating capacity using the Boyden Chamber assay (o). The peak of motility activity occurred between 900 and 1050 minutes, ~ 12% of the gradient.

Figure 2. Isolation of ATX by lectin affinity chromatography. 20 ml portions of the phenyl Sepharose activity peak were affinity purified on a 40 ml Concanavalin A Affi-Gel column. The bound components were eluted with a step gradient (----) of methyl α -D-mannopyranoside (0.0 mM, 10 mM, and 500 mM) in a buffer consisting of 0.05 M Tris (pH 7.5), 0.1 M NaCl, 0.01 M CaCl_2 and 20% ethylene glycol. Absorbance was monitored at 280 nm (_____) and indicated that the majority of the protein components did not bind to the column. Motility was assayed in 10 mL fractions (...o...) and was found predominantly in the 500 mM elution concentration. One of seven chromatographic runs is shown.

Figure 3. Purification of ATX by weak anionic exchange chromatography. Approximately 30% of the activity peak eluted from the Con A affinity column was applied to a ZORBAX BioSeries-WAX column. The bound components were eluted with an NaCl gradient (----) in a buffer consisting of 10 mM Tris (pH 7.5) and 30% ethylene glycol. Motility (o) was assayed in 1.0 ml fractions. The peak of activity eluted in a discrete but broad region in the shallow portion of the gradient. Absorbance was monitored at 230 nm (____). The majority of the protein components not associated with activity bound strongly to the column were eluted at 1.0 M NaCl. One of two chromatographic runs is shown.

Figure 4. Purification of ATX by molecular sieve exclusion chromatography. The entire activity peak eluted from the weak anion exchange column was applied to a series of TSK columns (4000SW, 4000SW, 3000SW, and 2000SW, in this order). Proteins were eluted in a buffer consisting of 0.1M NaPO_4 (pH 7.2) with 10% methanol and 10% ethylene glycol. Two major protein peaks were evident

Figure 5. Final purification of ATX by strong anionic exchange chromatography. Approximately 15% of the activity peak from the molecular sieve exclusion series was applied to a Pro-Pac PA1 column. Protein which bound to the column was eluted with a NaCl gradient (----) in a buffer consisting of 10 mM Tris (pH 7.5), 5% methanol and 20% ethylene glycol. Absorbance was monitored at 215 nM (____). Motility activity was assayed in 1.0 ml fractions at two different dilutions: 1/5 (...o....) or 1/15 (.__o.__). Activity was found to correspond to a double protein peak in the central region of the gradient.

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Figure 7. Two-dimensional gel electrophoresis of ATX. Purified ATX (Figure 6, Panel C) was subjected to non-equilibrium isoelectric focusing (5 hr. at 500v), then applied to a 7.5% SDS-polyacrylamide gel for the second dimension. The pH separation which resulted was measured in 0.5 cm samples of concurrently run tube gels and is shown at the top. Molecular weight standards for the second dimension are shown on the right. This analysis reveals a single component with $pI = 7.7 \pm 0.2$ and $M_r = 120,000$.

Figure 8. Dilution curve of ATX. Purified ATX (Figure 6, Panel C) was serially diluted and tested for motility-stimulating activity. The result, with unstimulated background motility subtracted out, shows that activity is half-maximal at ~ 500 pM ATX.

Figure 9. Pertussis toxin (PT) sensitivity of ATX. A2058 cells were pre-treated for 1 hr. prior to the start of the motility assay with $0.5 \mu\text{g/ml}$ PT in 0.1% BSA-DMEM or with 0.1% BSA-DMEM alone (for untreated control). The motility activity stimulated by purified ATX (Figure 6, Panel C) was then assessed for the two treatment groups. The result, expressed as cells/HPF \pm S.E.M. with unstimulated background motility subtracted out, reveals profound inhibition of PT-treated cells (hatched) compared to untreated cells (solid). PT had no effect on cell viability. S.E.M.'s were $< 10\%$.

Figure 10. Checkerboard analysis of ATX-stimulated motility. Varying dilutions of autotaxin were added to the upper chamber with the cells and/or to the lower chamber, as shown. Motility response, expressed as cells/HPF \pm S.E.M., was assessed for each point in the checkerboard.

Figure 11. Purification of ATX peptides on HPLC. ATX, purified to homogeneity by strong anionic exchange chromatography, was sequentially digested by cyanogen bromide, subjected to reduction and

pyridylethylation, and digested by trypsin. The resulting peptides were purified on an Aquapore RP300 C-8 reverse phase column using a (0-70)% acetonitrile gradient in 0.1% trifluoroacetic acid (----). The absorbance was monitored at 215 nm (____) and peaks were collected. Seven peaks, chosen at random for N-terminal amino acid sequence analysis, are shown with appropriate numbers.

Figure 12. Cloning Strategy, schematically depicted.

Figure 13. Schematic Diagram of autotaxin gene region.

For A2058: 4C11 is the original DNA clone obtained by screening an A2058 cDNA expression library in λ gt11 with anti-peptide ATX-102. Upstream ATX peptide sequences were utilized for PCR amplification of A2058 mRNA, using the technique of reverse transcription/PCR. These peptides include ATX-101, ATX-103, and ATX-224. The approximate localization of each of peptide was obtained by matching the peptide with its homologous region on PC-1.

For N-tera 2D1, a λ gt10 cDNA library was amplified and the cDNA inserts were isolated. PCR amplification, based on homologies with A2058 sequence, was utilized for DNA sequencing.

For normal human liver, a mRNA from liver was amplified with 5'RACE using primers from the known ATX-224 region of A2058 and N-tera 2D1.

Figure 14. Schematic match-up of ATX peptides with putative A2058 protein sequence.

Figure 15. Schematic match-up of ATX peptides with putative N-tera 2D1 protein sequence.

Figure 16: ATX Treatment with PNGase F. Partially purified ATX was treated with 60 mU/ml PNGase F at 37°C for 16 hr under increasingly denaturing conditions. The treated ATX samples were separated by SDS polyacrylamide gel electrophoresis run under reducing

conditions and stained with Coomassie blue G-250. Lane 1 contains untreated ATX (arrow) with no enzyme added. Lane 2 contains the reaction mixture run under non-denaturing conditions (50 mM tris/10% ethylene glycol, pH 7). Lanes 3 and 4 have added 0.1 M β -mercaptoethanol and 0.5% Nonidet-P40, respectively. Lanes 5 and 6 contain the reaction mixtures in which the ATX sample was first boiled for 3 min in 0.1% SDS with (lane 6) or without (lane 5) 0.1 M β -mercaptoethanol, then had 0.5% Nonidet-P40 added to prevent enzyme denaturation. The enzyme can be detected as an ~44 kDa band in lanes 2-6.

Figure 17: Effect of varying concentrations of PNGase F on ATX molecular weight and motility-stimulating activity. Partially purified ATX was treated with various concentrations (range 0 - 60 mU/ml, shown on horizontal axis) of PNGase F at 37°C for 16 hr. Figure 17A shows the effect of the different treatments on ATX molecular weight. At concentrations of enzyme ≥ 30 mU/ml, the deglycosylation reaction appears to be complete. Figure 17B shows the effect of the identical reaction mixtures on motility-stimulating capacity (immediately below the corresponding protein band of Figure 17A). There is no significant difference between any of the treatment groups.

Figure 18: Comparison of amino acid sequences of ATX and PC-1. The amino acid sequences of ATX and PC-1 are compared. Amino acid identity is indicated by a vertical line between the sequences. The location of the putative transmembrane/signal sequence is shown by a solid line. The two somatomedin B domains are identified by dashed lines. The putative phosphodiesterase active site is indicated by emboldened lines. The loop region of a single EF hand loop region is identified with double lines. The presumed cleavage site for each protein is indicated with arrows.

Figure 19: Domain structure of ATX and PC-1.

Putative domains are indicated for the two homologous proteins, ATX and PC-1.

DETAILED DESCRIPTION OF THE INVENTION

Tumor cell motility is a critical component of invasion and metatasis, but the regulation of this motility is still poorly understood. At least some tumor cells secrete autocrine motility factors (AMF's) that stimulate motility in the producing cells. Like the analogous autocrine growth factors, these AMF's allow tumor cells independence from the host in this important component of the metastatic cascade. One AMF, autotaxin (ATX), has recently been purified to homogeneity from the human melanoma cell line, A2058 (Stracke, et al., 1992). The purified protein was enzymatically digested and the peptide fragments were separated by reverse phase HPLC. A number of these peptides have been sequenced by standard Edman degradation (Table 6) from different purifications and different enzymatic digestion. Sequence information, obtained initially on 19 purified tryptic peptides, confirmed that the protein is unique with no significant homology to growth factors or previously described motility factors. These peptide sequences have now been used as the basis for identifying and sequencing the cDNA clone for ATX. The present invention comprises an amino acid sequence of ATX as well as a nucleic acid sequence coding for the ATX protein.

TABLE 6. PEPTIDE SEQUENCES FOR AUTOTAXIN.

PEPTIDE NO.	AMINO ACID SEQUENCE	SEQ ID: NO:
ATX-18	WHVAR	SEQ ID NO:1
ATX-19	PLDVYK	SEQ ID NO:2
ATX-20	YPAFK	SEQ ID NO:3
ATX-29	PEEVTRPNYL	SEQ ID NO:5

ATX-34B	RVWNYFQR	SEQ ID NO:38
ATX-41	HLLYGRPAVLY	SEQ ID NO:29
ATX-48	VPPFENIELY	SEQ ID NO:7
ATX-59	TFPNLYTFATGLY	SEQ ID NO:32
ATX-100	GGQPLWITATK	SEQ ID NO:8
ATX-101/223A	VNSMQTVFVGYGPTFK	SEQ ID NO:9
ATX-102	DIEHLTSLDFFR	SEQ ID NO:10
ATX-103	TEFLSNYLTNVDDITLVPETLGR	SEQ ID NO:11
ATX-104	VNVISGPIDDYDYDGLHDTEDK	SEQ ID NO:33
ATX-204	MHTARVRD	SEQ ID NO:39
ATX-205	FSNNAKYD	SEQ ID NO:40
ATX-209	VMPNIEK	SEQ ID NO:41
ATX-210	TARGWECT	SEQ ID NO:42
ATX-212	(N)DSPWT(N)ISGS	SEQ ID NO:43
ATX-214	LRSCGTHSPYM	SEQ ID NO:44
ATX-215/34A	TYLHTYES	SEQ ID NO:45
ATX-213/217A	AIIANLTCKKPDQ	SEQ ID NO:46
ATX-216	IVGQLMDG	SEQ ID NO:47
ATX-218/44	TSRSYPEIL	SEQ ID NO:48
ATX-223B/24	QAEVSSVPD	SEQ ID NO:49
ATX-224	RCFELQEAGPPDDC	SEQ ID NO:50
ATX-229	SYTSCCHDFDEL	SEQ ID NO:51
ATX-244/53	QMSYGFLFPPYLSSSP	SEQ ID NO:52

ATX is a glycosylated protein due to its high affinity for concanavalin A and amino acid sequence analysis of the ATX peptides. ATX has been demonstrated to be a 125kDa glycoprotein whose molecular weight reduced to 100-105kDa after deglycosylation with N-glycosidase F. The calculated molecular weight of the cloned protein is 100kDa (secreted form) or 105kDa (full length protein). Based on amino acid composition, the estimated pI is 9.0 which is higher than the pI determined by 2-D gel

electrophoresis analysis (7.7-8.0) of purified ATX. This difference can be explained by the presence of sialic acid residues on the sugar moieties.

Autotaxin is secreted by A2058 human melanoma cells cultured in low abundance in serum-free conditioned medium. Autotaxin is a potent new cytokine with molecular mass 125 kDa which has been purified to homogeneity from the conditioned medium of the human melanoma cell line, A2058, utilizing sequential chromatographic methods as described herein. This new cytokine, termed autotaxin (ATX), is a basic glycoprotein with pI ~ 7.8. ATX is active in the high picomolar to low nanomolar range, stimulating both chemotactic and chemokinetic responses in the ATX-producing A2058 cells as well as other tumor cells. This motile response is abolished by pretreatment of the cells with pertussis toxin. ATX may therefore act through a G protein-linked cell surface receptor. These characteristics distinguish ATX from several small growth factors and interleukins which are implicated in tumor cell motility (Stracke et al., 1988; Ruff et al., 1985; Maciag et al., 1984; Gospodarowicz, 1984; Van Snick, 1990; Yoshimura 1987).

The protein of the present invention, which in one embodiment is derived from A2058 human melanoma cells, can be prepared substantially free from proteins with which it is normally associated using, for example, the purification protocol disclosed herein. Alternatively, the protein of the present invention can be prepared substantially free from proteins, by cloning and expressing the cDNA encoding autotaxin as disclosed herein.

A large volume of serum-free conditioned medium from appropriate producer cells (e.g., tumor cells) is collected and concentrated approximately 500-fold. This concentrated conditioned medium is then separated from other contaminating proteins by techniques that rely on

the chemical and physical characteristics of the protein. These include the molecular weight, relative hydrophobicity, net charge, isoelectric focusing point, and the presence of lectin-binding sugar residues on the protein.

Alternatively, the protein, or functional portion thereof, can be synthesized using chemical or recombinant means.

The protein of the present invention has a potent biological activity. Purified ATX is active in the picomolar range and 1 unit of activity corresponds to a concentration of approximately 500 pM as assessed by the cell motility assay described herein and elsewhere (Stracke et al., 1989).

The protein of the present invention has a molecular size, as determined by two dimensional gel electrophoresis, of from 120 to 130 kDa, or more specifically, about 125 kDa. Further, the protein of the present invention can have a pI in the range of 7.5 to 8.0, preferably, approximately 7.7. The present invention relates to autotaxin and peptides thereof having cell motility properties as described herein. These proteins and peptides thereof can be produced by isolation from a natural host or isolation as an expression product from a recombinant host.

The present invention also relates to a DNA segment coding for a polypeptide comprising an amino acid sequence corresponding to ATX, or a unique portion of such a sequence (unique portion being defined herein as at least 5, 10, 25, or 50 amino acids). In one embodiment, the DNA segment encodes any one of the amino acid sequences shown in SEQ ID NO:1 to SEQ ID NO:11 and SEQ ID NO:26 to SEQ ID NO:33. Another embodiment uses larger DNA fragments encoding amino acid sequences shown in SEQ ID NO:34, SEQ ID NO: 36 and SEQ ID NO:38. The entire coding region for autotaxin can also be used in the present invention shown

in SEQ ID NO:66 through SEQ ID NO:69.

In another embodiment, the present invention relates to a recombinant DNA molecule comprising a vector (for example plasmid or viral vector) and a DNA segment coding for a polypeptide corresponding to ATX, as can be prepared by one skilled in the art. Preferably, the coding segment is present in the vector operably linked to a promoter. The present invention also relates to a recombinant protein produced from a host cell expressing a cDNA containing a coding region of ATX. Examples of ATX cDNAs from a variety of sources have been cloned and can be used for expression, including *inter alia* A2058 carcinoma cells, N-tera 2D1 cells and human liver.

In a further embodiment, the present invention relates to a cell containing the above-described recombinant DNA molecule. Suitable host cells include procaryotic cells (such as bacteria, including E. coli) and both lower eucaryotic cells (for example, yeast) and higher eucaryotic cells (for example, mammalian cells). Introduction of the recombinant molecule into the host cells can be effected using methods known in the art.

In another embodiment, the present invention relates to a method of producing a polypeptide having an amino acid sequence corresponding to ATX. The method comprises culturing the above-described cells under conditions such that the DNA segment is expressed, and isolating ATX thereby produced.

In a further embodiment, the present invention relates to an antibody having affinity for ATX or peptide fragments thereof. The invention also relates to binding fragments of such antibodies. In one preferred embodiment, the antibodies are specific for ATX peptides having an amino acid sequence set forth in one of SEQ ID NO:1 through SEQ ID NO:11 and SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO: 36 and SEQ ID NO:38 through SEQ ID NO:52. In addition, the antibodies may recognize an

entire autotaxin protein.

Antibodies can be raised to autotaxin or its fragment peptides, either naturally-occurring or recombinantly produced, using methods known in the art.

The ATX protein and peptide fragments thereof described above can be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as carrier proteins. In particular, ATX fragments can be fused or covalently linked to a variety of carrier proteins, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See for example, Harper and Row, (1969); Landsteiner, (1962); and Williams et al., (1967), for descriptions of methods of preparing polyclonal antisera. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and the gamma globulin is isolated.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts. Description of techniques for preparing such monoclonal antibodies may be found in Stites et al., and references cited therein, and in particular in Kohler and Milstein (1975), which discusses one method of generating monoclonal antibodies.

In another embodiment, the present invention relates to an oligonucleotide probe synthesized according to the sense or antisense degenerative sequence set forth in one of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:33, SEQ ID NO:39 through SEQ ID NO:52, and SEQ ID NO:55 through SEQ ID NO:65.

Protein database searches of this sequence revealed a 45% amino acid identity with the plasma cell membrane marker protein, PC-1. ATX and PC-1 appear to share a number of domains, including two somatomedin B domains, the loop region of an EF hand, and the enzymatic site of

type I phosphodiesterase/ nucleotide pyrophosphatase. Like PC-1, ATX hydrolyzes p-nitrophenyl thymidine-5' - monophosphate, a type 1 phosphodiesterase substrate. This enzymatic function of ATX suggests a newly identified function for ecto/exo-enzymes in cellular motility.

In a further embodiment, the present invention relates to a method of diagnosing cancer metastasis and to a kit suitable for use in such a method. Preferably, antibodies to ATX can be used in, but not limited to, ELISA, RIA or immunoblots configurations to detect the presence of ATX in body fluids of patients (e.g. serum, urine, pleural effusions, etc.). These antibodies can also be used in immunostains of patient samples to detect the presence of ATX.

In yet another embodiment, the present invention relates to *in vivo* and *in vitro* diagnostics. ATX may be radiolabelled, by means known to one skilled in the art, and injected in cancer patients with appropriate ancillary substances also known to one skilled in the art, in order to ultimately detect distant metastatic sites by appropriate imagery. The level of ATX in tissue or body fluids can be used to predict disease outcomes and/or choice of therapy which may also include ATX inhibitors.

In a further embodiment, the present invention relates to a treatment of cancer. ATX antibodies can be cross-linked to toxins (e.g., Ricin A), by means known to one skilled in the art, wherein the cross-linked complex is administered to cancer patients with appropriate ancillary agents by means known to one skilled in the art, so that when the antibody complex binds to the cancer cell, the cell is killed by the cross-linked toxin.

In another embodiment, the different localizations of the normal versus tumorous forms of the ATX proteins within the tissue can be used as a tool for diagnosis and prognosis. The stage of disease progression can be monitored by elevated levels of ATX in the extracellular

space as opposed to its normal cell membranes association. In addition, treatment methods for control of tumor progression can be designed to specifically block the activity of the secreted form of ATX. Such methods would have a preferential effect upon secreted ATX during tumor progression while not effecting normal ATX formation.

Yet another embodiment utilizes the hot spot located in the region from approximately nucleotides 1670 through 1815, as a marker gene for identification of tissues carrying a tumorous form of ATX.

The present invention is described in further detail in the following non-limiting examples.

EXAMPLES

The following protocols and experimental details are referenced in the Examples that follow:

Materials. The polycarbonate Nuclepore membranes and the 48-well microchemotaxis chambers were obtained from Neuro Probe, Inc. Pertussis toxin (PT), ethylene glycol (biotechnology grade), methyl α -D-mannopyranoside were obtained from commercial vendors. The ampholyte, pH 3-10 Bio-Lyte and pH 8-10 Bio-Lyte, were obtained from Bio-Rad. Phenyl Sepharose CL-4B; affi-Gel concanavalin A; ZORBAX BioSeries-WAX (weak anion exchange) column (9.4mm x 24cm); Spherogel-TSK 4000SW, 3000SW and 2000SW columns (each 7.5mm x 30cm); the Pro-Pac PA1 (4 x 50mm) strong anion exchange column; the Aquapore RP300 C-8 reverse phase column (220 x 2.1mm); and the AminoQuant C-18 reverse phase column (200 x 2.1mm) were also obtained from commercial sources.

Affi-Gel 10 affinity resin was from Bio-Rad. The GeneAmp PCR Reagent kit with AmpliTaq and the GeneAmp RNA PCR kit were purchased from Perkin-Elmer. The 5' RACE kit came from Gibco BRL Life Technologies, Inc. The p-nitrophenyl thymidine-5'monophosphate was obtained from Calbiochem Biochemicals.

Ethylene glycol (biotechnology grade) was from Fisher

Biochemicals (Pittsburg, PA). Peptide N-glycosidase F ("PNGase F"), O-glycosidase, neuraminidase (*Arthrobacter ureafaciens*), and swainsonine ("Swn") came from Boehringer-Mannheim (Indianapolis, IN). 1-Deoxymannojirimycin ("dMAN"), and N-methyl-1-deoxynojirimycin ("NMdNM") were from Oxford GlycoSystems, Inc. (Rosedale, NY). Biotinylated concanavalin A, HRP-conjugated streptavidin, and HRP-conjugated goat anti-rabbit immunoglobulin were purchased from Pierce Chemicals (Rockford, IL). Polyvinyl pyrrolidone-free polycarbonate membranes and the microchemotaxis chamber were from NeuroProbe, Inc. (Cabin John, MD).

Cell Culture. The human melanoma cell line A2058, originally isolated by Todaro (Todaro et al., 1980), was maintained as previously described by Liotta (Liotta et al., 1986). The N-tera 2 (D1 clone) was a kind gift from Dr. Maxine Singer, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health and was maintained as described (Andrews, P.W., Goodfellow, P.N. and Bronson, D.L. (1983) *Cell surface characteristics and other markers of differentiation of human teratocarcinoma cells in culture.*).

Production of Autotaxin. A2058 cells were grown up in T-150 flasks, trypsinized, and seeded into 24,000 cm² cell factories at a cell density of 1×10^{10} cells/factory. After 5-6 days, the serum-containing medium was removed and the cells were washed with DPBS. The factories were maintained in DMEM without phenol red, supplemented with 4 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 5 µg/ml crystallized bovine serum albumin, 10 µg/ml bovine insulin, and 1 µM aprotinin. Culture supernatants were harvested every 3 days, frozen at -40°C and replaced with fresh serum-free medium. Each cycle of supernatant was tested for ATX production with a cell motility assay detailed below. Typically, a cell factory continued to be productive for 9-11 of these cycles.

After accumulation of approximately 45-60 L of supernatant, the culture supernatants were thawed and concentrated down to 2-2.5 L using an Amicon S10Y30 spiral membrane ultrafiltration cartridge. This supernatant was further concentrated in an Amicon high performance ultrafiltration cell using Diaflo membranes. The final volume achieved from 100-200 L of conditioned medium was typically 250-400 ml. All ultrafiltrations were performed at 4°C.

Cell Motility Assays. Purification of autotaxin was monitored by testing the motility-stimulating capacity of the fractions collected from the columns. These fractions were in buffers unsuitable for a chemotaxis assay so each fraction had to be washed into an appropriate buffer, i.e., 0.1% (w/v) BSA in DPBS containing calcium and magnesium. This dialysis was performed by adding aliquots of each fraction to be tested into Centricon-30™ ultrafiltration tubes, which retain molecular species larger than 30,000 daltons.

The assay to determine motility was performed in triplicate using a 48-well microchemotaxis chamber as described elsewhere in detail (Stracke et al., 1987; Stracke, et al., 1989). The Nuclepore™ membranes used in these modified Boyden chambers were fixed and stained with Diff-Quik.™ Chemotaxis was quantitated either by reading the stained membranes with a 2202 Ultrosan laser densitometer or by counting 5 randomly chosen high power fields (HPF) under light microscopy (400 x) for each replicate. Densitometer units (wavelength - 633 nm) have been shown to be linearly related to the number of cells per HPF (Taraboletti, 1987; Stracke, et al., 1989). Typically, unstimulated motility (background) corresponded to 5-10 cells/HPF and highly responding cells to 70-100 cells/HPF above unstimulated background (i.e., 75-110 total cells/HPF).

For experiments using PT, the toxin was pre-incubated

3 ml/min.

The active peak was pooled, dialyzed against 0.1 M sodium phosphate (pH 7.2), 10% (v/v) methanol, and 10% (v/v) ethylene glycol, and subjected to a fourth fractionation step on a series of Spherigel TSK columns (4000SW, 4000SW, 3000SW, 2000SW, in that order). This molecular sieve step was run using the Shimadzu BioLiquid chromatograph at 0.4 ml/min.

The active peak was pooled and dialyzed into 10 mM Tris (pH 7.5), 5% (v/v) methanol, 20% (v/v) ethylene glycol and subjected to a fifth (strong anion exchange) chromatography step, a Pro-Pac PA1 column run at 1 ml/min using a Dionex BioLC with AI450 software. The sample was eluted with a linear gradient of (0.0-0.4M) NaCl.

In order to calculate activity yields after each step of purification, a unit of activity had to be derived. The dilution curve of ATX was biphasic with a broad peak and a linear range at sub-optimal concentrations. One unit of activity/well (i.e., 40 units/ml) was defined as 50% of the maximal activity in a full dilution curve. This allowed calculation of the activity contained in any volume from the dilution needed to achieve 1 unit/well. Therefore, if a 1:10 dilution were needed in order to produce 1 unit of activity/well, the material contained $10 \times 40 = 400$ units/ml.

Gel Electrophoresis. Protein samples were analyzed by SDS-polyacrylamide gel electrophoresis using the conditions of Laemmli (Laemmli, 1970). In brief, 7 or 8% SDS-containing polyacrylamide gels were prepared or pre-poured (8-16%) gradient gels were obtained commercially. Samples were prepared with or without reducing conditions (5% β -mercaptoethanol). After electrophoretic separation, the gels were stained using Coomassie Blue G-250 as previously described (Neuhoff, et al., 1988). In this staining protocol, which ordinarily requires no destaining step, the Coomassie stain appears to be able to stain as little

as 10 ng of protein.

For two-dimensional electrophoresis, the protein, in 20% ethylene glycol, was dried in a Speed-vac and redissolved in loading solution: 9M urea, 1% (v/v) pH 3-10 Bio-Lyte, and 2.5% (v/v) Nonidet-P40. This sample was then subjected to isoelectric focusing (O'Farrell, 1975) using a Bio-Rad tube cell in 120 x 3 mm polyacrylamide tube gels containing 9M urea, 2% (v/v) pH 3-10 Bio-Lyte, 0.25% (v/v) pH 8-10 Bio-Lyte and 2.5% (v/v) Nonidet-P40. Reservoir solutions were 0.01 M phosphoric acid and 0.02 M NaOH. Non-equilibrium isoelectric focusing (O'Farrell, et al., 1977) was run initially with constant voltage (500 v) for 5 hr. Since the protein was basic, the procedure was repeated under equilibrium conditions (500 v for 17 hr.). Electrophoresis in the second dimension was performed on a 7.5% polyacrylamide using the conditions of Laemmli (1970). The gel was stained with Coomassie Blue G-250 as above.

Preparation of peptides for internal sequence of autotaxin. Homogeneous ATX was sequentially digested with cyanogen bromide and, following reduction and pyridylethylation, with trypsin (Stone, et al., 1989). The resulting fragments were then separated by gradient elution on an Aquapore RP300 C-8 reverse phase column: 0.1% (v/v) trifluoroacetic acid and (0-70)% acetonitrile over 85 min. at a flow rate of 0.2 ml/min. A Dionex AI450 BioLC system was utilized and fractions were collected manually while monitoring the absorbance at 215 nm.

Sequence analysis of peptides. The amino acid sequences of peptides resulting from digestion and purification of ATX peptides #1-7 and 12-18, corresponding to SEQ ID NO:1 through SEQ ID NO:7 and SEQ ID NO:26 through SEQ ID NO:32, respectively, were determined on a Porton Instruments 2020 off-line sequenator using standard program #1.

Phenylthiohydantoin amino acid analysis of sequenator runs were performed on a Beckman System Gold HPLC using a

modified sodium acetate gradient program and a Hewlett-Packard C-18 column. ATX-100 (SEQ ID NO:8), ATX-101 (SEQ ID NO:9), ATX-102 (SEQ ID NO:10), ATX-103 (SEQ ID NO:11) and ATX 104 (SEQ ID NO:33) were sequenced from gel-purified ATX.

Protein databases (Pearson, et al. 1988) that were searched for homologies in amino acid sequence with the ATX peptides include: GenBank (68.0), EMBL (27.0), SWISS-PROT (18.0), and GenPept (64.3).

EXAMPLE 1

Purification of Autotaxin

The A2058 cells had been previously shown to produce protein factors which stimulate motility in an autocrine fashion (Liotta, et al., 1986). Conditioned medium from these cells was therefore used to identify and purify a new motility-stimulating factor, which is here named autotaxin and referred to as ATX. Since the purification was monitored with a biological assay, motility-stimulating activity had to be maintained throughout. The activity proved to be labile to freezing, acidic buffers, proteases (but not DNase or RNase), reduction, strong chaotropic agents (e.g. > 4 M urea), and a variety of organic solvents (isopropanol, ethanol, acetonitrile). An organic solvent, ethylene glycol, which did not decrease bioactivity, was added for both storage and chromatographic separation.

100-200 L of serum-free conditioned medium were required in order to produce enough ATX for amino acid sequence analysis. The medium contained low concentrations of both BSA (5 μ g/ml) which was needed as a carrier protein and insulin (10 μ g/ml) which was required to support cell growth in low protein medium. Ultrafiltration to concentrate this large volume was performed with low protein-binding YM30 membranes which retain molecular species with M_r > 30,000. As seen in Table 1, 200 L of conditioned medium prepared in this

manner resulted in 10×10^6 units of activity. However, the initial unfractionated conditioned medium contained additional substances known to stimulate activity, particularly insulin, which does not completely wash out in the ultrafiltration step and which is additive to the motility stimulating activity in a complex manner (Stracke, et al., 1989). This had to be taken into account in order to determine yields for subsequent steps in which insulin had been removed.

TABLE 1. PURIFICATION OF AUTOTAXIN

Purification Step Recovery	Protein (mg)	Activity ^a (total units)	Specific	
			Activity (units/mg)	(%) ^b
200 L Conditioned Medium	33,000	10,000,000 ^c	300	
Phenyl Sepharose	1,235	460,000	370	100
Concanavalin A	58	660,000	11,400	100
Weak Anion Exchange	4.5	490,000	110,000	100
TSK Molecular Sieves	~ 0.4 ^d	220,000	550,000	48
Strong Anion Exchange	~ 0.04 ^d	24,000 ^c	600,000	5.2

^a Activity calculated from Boyden chamber assay. The dilution which resulted in 50% of maximal activity (generally approximately 20 laser density units or ~ 40 cells/HPF) was chosen to have 1 unit of activity per well (equivalent to 40 units/ml).

^b Recovery was estimated from activity, after the first purification column (i.e., phenyl sepharose).

^c Initial activity in the unfractionated conditioned medium reflected the fact that insulin was used in the medium as a necessary growth factor under low protein conditions.

- ^d Estimated protein is based on quantification by amino acid analysis.
- ^e This specific activity for purified protein corresponds to ~ 10 fmol ATX/unit of motility activity (in a Boyden chamber well).

The first step in the purification involved fractionation by hydrophobic interaction chromatography using a phenyl Sepharose CL-4B column. The results are shown in Figure 1. Most proteins, including insulin, eluted from the column in early fractions or in the void. However, the peak of activity eluted relatively late. The activity which was purified was estimated as 460,000 units \pm 20% (Table 1). As the pooled peak of activity from the phenyl Sepharose fractionation is considered to be the first sample without significant insulin contamination, subsequent yields are measured against its total activity. Gel electrophoresis of a small portion of the pooled peak of activity (Figure 6A, column 2) revealed a large number of protein bands with BSA predominant from the original conditioned medium.

In the second step of purification, the active peak was applied to the lectin affinity column, Affi-Gel concanavalin A. As shown in Figure 2, most protein (estimated to be 90% of the total absorbance at 280 nm) failed to bind to the column at all. The non-binding fraction contained essentially no motility-stimulating activity (see dotted line in Figure 2). When a linear gradient of methyl α -D-mannopyranoside was applied to the column, chemotactic activity eluted off in a prolonged zone, beginning at a concentration of approximately 20 mM sugar. Consequently, a step gradient was used to elute. Pure BSA failed to bind to con A.

Activity was found primarily in the 500 mM step of methyl α -D-mannopyranoside. There appeared to be no significant loss of activity as seen in Table 1; however, specific activity (activity/mg total protein) increased thirty-fold. Gel electrophoresis of the pooled and

concentrated peak (Figure 6A, column 3) revealed that the BSA overload was no longer apparent and the number of bands were much reduced. When the unbound protein was concentrated and applied to a gel, it appeared identical to the active peak from phenyl Sepharose-4B with a large BSA band.

The third purification step involved fractionating the previous active peak by weak anion exchange chromatography as shown in Figure 3. Under the running conditions, activity eluted in a broad peak-shoulder or double peak in the middle of the shallow portion (0.0-0.4 M) of the NaCl gradient. The largest proportion of protein, lacking in motility-stimulating capacity, bound strongly to the column and eluted off in high salt (1 M NaCl). There appeared to be no significant loss of activity, though specific activity increased by twenty-fold (Table 1). Analysis by gel electrophoresis of both the peak (28-34 min. in Figure 3) and the shoulder (35-45 min. in Figure 3) is shown in Figure 6A (columns 4 and 5, respectively). Two predominant protein bands resulted: a broad doublet around 25-35 kDa and a second doublet around 110-130 kDa.

In the fourth purification step, the active peak was applied to a series of molecular sieves. Spectrophotometric monitoring of the eluant revealed two large peaks of protein (Figure 4). Activity corresponded to the first, higher molecular weight peak. Recovery of activity was ~48% with a five-fold increase in specific activity. Analysis by gel electrophoresis was performed under non-reducing and reducing conditions as shown in Figure 6B (columns 2 and 3, respectively). This fractionation step had essentially removed all contaminating protein of molecular weight < 55 kDa. The predominant band remaining has a molecular weight of 120 kDa unreduced and 125 kDa reduced; there are two minor bands with molecular weights 85 kDa and 60 kDa. The fact

that the 120 kDa protein changes so little in electrophoretic mobility after reduction would tend to indicate a paucity of disulfide bonds. However, the existing disulfide bonds have functional significance because motility-stimulating activity is labile to reduction.

The fifth purification step involved fractionation of the active peak by strong anion exchange chromatography. As shown in Figure 5, activity corresponds to two broad optical absorbance peaks in the middle of the gradient with contaminating proteins eluting earlier. These two peaks were identical by amino acid analysis and by polyacrylamide gel electrophoretic separation. They presumably represent different glycosylation states of the same parent protein. Activity is shown in Figure 5 at two different sample dilutions. Several dilutions of the fractionated samples were often necessary in order to resolve the true "peak" of activity as the shape of the ATX dilution curve was not sharp due to saturation and down regulation at high concentrations. Recovery from this chromatographic step is lower (5% compared to phenyl Sepharose), as might be expected when a minute quantity of protein is applied to a column; however, specific activity again increased (Table 1). Analysis by gel electrophoresis revealed a single protein band at molecular weight 120 kDa, unreduced (Figure 6C, column 2).

EXAMPLE 2

Characterization of Autotaxin

Two dimensional gel electrophoresis of the purified protein (Figure 7) revealed a single predominant band. The band slopes downward slightly toward the basic side of the gel in a manner that is characteristic of glycosylated proteins. A basic pI of 7.7 ± 0.2 was essentially the same whether the isoelectric focusing was run under non-equilibrium conditions (5 hr.) or was allowed to go to equilibrium (17 hr.).

A dilution curve of the purified protein is shown in Figure 8. The protein is active in the picomolar range and 1 unit of activity appears to correspond to a concentration of 400-600 picomolar (or approximately 10 fmol of ATX/Boyden chamber well). When dilutions were begun at higher concentrations of ATX, the resultant curve showed a broad plateau with down-regulation at the highest concentrations. The motility response to purified autotaxin is highly sensitive to pertussis toxin (hereinafter referred to as "PT") (Table 2 and Figure 9) with approximately 95% inhibition of activity at 0.5 μ g/ml PT.

TABLE 2. Effect of Pertussis Toxin (PT) on Autotaxin-stimulated motility

	A2058 Motility Response (density units ¹)	
	control cells ²	Pertussis toxin-treated cells ³
Condition medium ⁴	60.3	0.4
Purified Autotaxin	38.5	0.0

¹ Chemotaxis quantitated by motility assay (Stracke, et al., 1978).

² A2058 cell suspended at 2×10^6 cells/ml in DMEM supplemented with 1 mg/ml bovine serum and rocked at room temperature for 1 hr.

³ As control with 0.5 μ g/ml pertussis toxin.

⁴ Prepared by adding DMEM without phenol red supplemented with 0.1 mg/ml bovine serum albumin to subconfluent flasks of A2058 cells. The medium was harvested after 2 days incubation at 37°C in a humidified atmosphere and concentrated 25-30 fold using an Amicon ultrafiltration assembly with a YM-30 membrane.

Checkerboard analysis was performed to assess the random (chemokinetic) versus the directed

(chemotactic) nature of the motility response to ATX. Chambers were assembled with different concentrations of ATX above and below the filter, using ATX purified through the weak anion exchange fractionation step. Squares below the diagonal reflect response to a positive gradient, squares above reflect response to a negative gradient, and squares on the diagonal reflect random motility in the absence of a gradient. ATX stimulates both chemotactic and chemokinetic responses (Figure 10), with chemotactic responses as high as fifteen-fold above background and chemokinesis as high as eight-fold above background.

Amino acid analysis after complete acid hydrolysis was used to quantitate purified protein. This hydrolysis was carried out on protein excised from a polyacrylamide gel and presumed to be pure. The analysis indicated that 2.7 nmol of protein was present after fractionation on the molecular sieve. After fractionation by strong anion exchange chromatography, approximately 300 pmol remained. The results of the analysis are shown in Table 3.

TABLE 3. AMINO ACID COMPOSITION OF AUTOTAXIN
(CYS and TRP were not determined in this analysis)

<u>Amino Acid</u>	<u>Residues/100</u>
ASX	12.5
THR	6.0
SER	5.7
GLX	9.4
PRO	7.4
GLY	7.0
ALA	3.9
VAL	6.7
MET	1.2
ILE	4.3
LEU	9.0
TYR	5.2
PHE	5.2
HIS	3.8
LYS	7.4
ARG	5.4

EXAMPLE 3

ATX Degradation and Determination of
Amino Acid Sequence

Attempts to obtain N-terminal sequence information from purified ATX repeatedly proved futile. The purified protein was therefore, sequentially digested and the resulting peptides fractionated by reverse phase chromatography. The results are shown in Figure 11. Multiple sharp peaks including clusters at both the hydrophilic and hydrophobic ends of the gradient are seen.

Several of these peptide peaks were chosen randomly for Edman degradation and N-terminal amino acid sequence analysis. Seven of the eight peaks (shown in Figure 11) chosen gave clear single sequence information as seen in Table 4. Using material from a separate digestion and purification, the remaining four sequences were also obtained.

Separate sense and antisense oligonucleotide probes were synthesized according to the fragment sequences of Table 4 by methods known to one skilled in the art. Representative probes are shown in Table 5.

TABLE 4. Peptide sequences for Autotaxin.

PEPTIDE NO.	AMINO ACID SEQUENCE	SEQ ID: NO:	NAME
1.	WHVA	SEQ ID NO:1	ATX 18
2.	PLDVYK	SEQ ID NO:2	ATX 19
3.	YPAFK	SEQ ID NO:3	ATX 20
4.	QAEVS	SEQ ID NO:4	ATX 24
5.	PEEVTRPNYL	SEQ ID NO:5	ATX 29
6.	YDVPWNETI	SEQ ID NO:6	ATX 47
7.	VPPFENIELY	SEQ ID NO:7	ATX 48
8.	GGQPLWITATK	SEQ ID NO:8	ATX 100

9.	VNSMQTVFVGY- GPTFK	SEQ ID NO:9	ATX 101
10.	DIEHLTSLDFFR	SEQ ID NO:10	ATX 102
11.	TEFLSNYLTVDD- ITLVPETLGR	SEQ ID NO:11	ATX 103
12.	QYLHQYGSS	SEQ ID NO:26	ATX 37
13.	VLNYF	SEQ ID NO:27	ATX 39
14.	YLNAT	SEQ ID NO:28	ATX 40
15.	HLLYGRPAVLY	SEQ ID NO:29	ATX 41
16.	SYPEILTPADN	SEQ ID NO:30	ATX 44
17.	XYGFLFPPYLSSSP	SEQ ID NO:31	ATX 53
18.	TFPNLYTFATGLY	SEQ ID NO:32	ATX 59
19.	VNVISGPIFDYDYDGLH DTEDK	SEQ ID NO:33	ATX 104

Peptide numbers 1-7 refer to peaks numbered in Figure 11. Peptide numbers 12-18 refer to peptides purified from the preparation which yielded peptide numbers 1-7. Peptides 8-11 and 19, are from a separate purification, not shown in Figure 11.

X refers to potentially glycosylated residues.

TABLE 5.

Oligonucleotides synthesized from peptide sequences of autotaxin (ATX). The number of the oligonucleotide corresponds to the ATX peptide number as per Table 4. The final letter suffix distinguishes whether the oligonucleotide is a sense (S) or antisense (A) sequence.

<u>Oligo</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>
A-18A	GTT-GGC-AGC-NAC-RTG-CCA	SEQ ID NO:12
A-18S	TGG-CAY-GTN-GCT-GCC-AAC	SEQ ID NO:13
A-20A	CTT-GAA-GGC-AGG-GTA	SEQ ID NO:14
A-20S	TAY-CCT-GCN-TTY-AAG	SEQ ID NO:15
A-29A	GGT-NAC-YTC-YTC-AGG	SEQ ID NO:16

A-29S	CCT-GAR-GAR-GTN-ACC	SEQ ID NO:17
A-47A	NGT-NGC-RTC-RAA-TGG-CAC-RTC	SEQ ID NO:18
A-47S	GAY-GTG-CCA-TTY-GAY-GCN-ACN	SEQ ID NO:19
A-48A	GTT-DAT-RTT-STC-RAA-TGG-GGG	SEQ ID NO:20
A-48S	CCC-CCA-TTT-GAG-AAC-ATC-AAC	SEQ ID NO:21
A-100A	CTT-NGT-NGC-NGT-DAT-CCA-NAR- GGG-YTG-GCC-GCC	SEQ ID NO:22
A-100S	GGC-GGC-CAR-CCC-YTN-TGG-ATH- ACN-GCN-ACN-AAG	SEQ ID NO:23
A-101A	CTT-RAA-GGT-GGG-GCC-RTA-GCC- CAC-RAA-GAC-TGT-YTG-CAT	SEQ ID NO:24
A-101S	ATG-CAR-ACA-GTC-TTY-GTG-GGC- TAY-GGC-CCC-ACC-TTY-AAR	SEQ ID NO:25

EXAMPLE 4

Antipeptide Antibodies

Rabbits were injected with ATX-101 (SEQ ID NO:10) which had been cross-linked to bovine serum albumin. Antisera from these rabbits was subjected to salt precipitation followed by purification using affinity chromatography with Affi-Gel 10 beads covalently linked to the peptide, ATX-101 (SEQ ID NO:10). This affinity purified antibody reacted with the partially purified protein on immunoblots. This same antibody has been used to perform immunohistochemical stains on human tissue.

EXAMPLE 5

Enzymatic Deglycosylation of ATX

Purified ATX that was to be treated with peptide N-glycosidase F (PNGase F) was first dialyzed into 0.2 M sodium phosphate, 10% (v/v) ethylene glycol pH 7.0, using Centricon-30 ultrafiltration tubes. Varying concentrations of PNGase F were added to the ATX and incubated 16-18 hr. at 37°C. Complete digestion appeared

to occur at concentrations of enzyme above 30 mU/ml (where 1 U converts 1mmol of substrate/min). For comparison, the experiments were repeated in the presence of 0.1 M β -mercaptoethanol or 0.1% (w/v) SDS plus 0.5% (v/v) Nonidet-P40. ATX that was to be treated with neuraminidase or O-glycosidase was dialyzed into 20 mM sodium phosphate, 0.1 M calcium acetate, and 10% (v/v) ethylene glycol (pH 7.2). Neuraminidase was added to a final concentration of 2 U/ml. For treatment with neuraminidase alone, this mixture was incubated 16-18 hr at 37°C. Since O-glycosidase requires the removal of terminal sialic acid residues for efficient deglycosylation, ATX was pre-incubated with neuraminidase for 30-125 mU/ml and incubated 16-18 hr. at 37°C. The treated ATX was then dialyzed into 50 mM Tris with 20% ethylene glycol for storage at 5°C.

Treatment of ATX with N-glycosylation altering agents

A2058 cells were split into four 150 cm² flasks and incubated until just subconfluent in DMEM supplemented with 10% fetal calf serum. The medium was then replaced with fresh 10% FCS/DMEM to which had been added DPBS for control, 1mM dMAN, 1 mM NMdNM, or 10 mM (1.7 mg/ml) Swn. Concentrations of these pharmacological agents were similar to those previously described as inhibiting N-glycan processing enzymes in melanoma cells (Seftor, et al. 1991; Dennis, et al. 1990) as well as carcinoma cells (Ogier, et al. 1990). On the next day, each flask was washed twice with Dulbecco's phosphate buffered saline with calcium ("DPBS") then 20 ml of Dulbecco's minimum essential medium ("DMEM") supplemented with 0.01% (w/v) bovine serum albumin ("BSA") was added. The same concentration of each agent was added to the appropriate equilibrated flask and incubated for ~ 24 hr, after which the medium from each treatment group was collected, concentrated, washed into DPBS and stored at 5°C.

Cells from each flask were trypsinized and

counted. There was no loss of viability or reduced cell number in any of the treatment groups compared to control cells.

Effect of PNGase F on ATX

ATX binds to concanavalin A ("Con A") agarose beads and is eluted with buffer containing 0.5 M methyl α -D-mannopyranoside, indicating that ATX is likely to contain mannose residues. Such mannose sugar residues are most characteristic of N-linked oligosaccharides. In order to verify that ATX contained asparagine-linked oligosaccharides, we treated it with the endoglycosidase, PNGase F, which cleaves high mannose, hybrid, and complex N-linked oligosaccharides at the asparagine residue.

Partially purified ATX was treated with 60 mU/ml of enzyme under a variety of increasingly denaturing conditions and then separated by polyacrylamide gel electrophoresis (Figure 16). Lane 1 shows untreated material; the 125 kDa band (arrow) is autotaxin. When this material is treated overnight with PNGase F under very mild conditions, the size of the 125 kDa band decreases to ~100- 105 kDa. Addition of 0.1 M β -mercaptoethanol (Lane 2) or 0.5% Nonidet-P40 (lane 3) to the ATX sample has no effect on the size of the resultant protein band. Even complete denaturation of ATX of boiling the sample for 3 min in 0.1% SDS with (lane 5) or without (lane 4) β -mercaptoethanol, followed by addition of 0.5% Nonidet-P40 to maintain enzymatic activity, has no effect on the final size of deglycosylated protein, indicating that the deglycosidation reaction was complete even under mild conditions.

Because these results showed that ATX contained N-linked oligosaccharide groups, it became important to see if these sugar moieties were necessary for stimulation of motility. The partially purified ATX sample was treated with varying concentrations of PNGase F (0.1 to 60 mU/ml) under mild, non-denaturing conditions. Analysis of

the resulting digest by polyacrylamide gel electrophoresis is shown in Figure 17A. As this figure shows, the digestion was incomplete using from 0.1 to 10 mU/ml of enzyme and resulted in a smear of protein between 100-125 kDa. However, at higher concentrations of enzyme, cleavage of N-linked oligosaccharides from ATX appears to be complete. When these different digestion products were compared for their capacity to stimulate motility (Figure 17B), there was no significant difference between groups.

EXAMPLE 6

Cloning the 3' end of Autotaxin (4C11)

ATX is active in picomolar to nanomolar concentrations and is synthesized in very small concentrations by A2058 cells. As might be expected, the cDNA clone was relatively rare, requiring various strategies and multiple library screenings in order to identify it (Figure 12). Attempts to utilize degenerate oligonucleotides deduced from known peptide sequences were unsuccessful--whether we used the oligo nucleotides for screening cDNA libraries or for reverse transcription of mRNA followed by amplification with the polymerase chain reaction (RT/PCR). We then utilized an affinity-purified anti-peptide ATX-102 antibodies to screen an A2058 expression library.

These anti-peptide antibodies were generated by methods well established in the art and described previously with slight modification (Wacher, et al., 1990). In brief, the previously identified peptide, ATX-102 (Stracke, et al., 1992), was synthesized on a Biosearch 9600 peptide synthesizer. It was then solubilized in 1X PBS containing 20% (v/v) DMSO and conjugated to the protein carrier, bovine serum albumin (BSA), with glutaraldehyde. For the first injection into New Zealand white rabbits, the BSA-peptide conjugate was emulsified with complete Freund's adjuvant and injected subcutaneously. For subsequent injections, the BSA-

peptide conjugate was emulsified with incomplete Freund's adjuvant. The resultant antiserum was heat-inactivated at 56°C for 30 min. Immunoglobulins were precipitated out in 47% saturated ammonium sulfate, then redissolved and dialyzed into PBS. Antibodies were adsorbed onto peptide-conjugated Affi-Gel 10 resin (made using the BioRad protocol), eluted with 0.1 N acetic acid, and neutralized with 2 M Tris-HCl, pH 8. The resulting affinity-purified antibodies were dialyzed into DPBS, concentrated, aliquotted, and stored at -20°C. The antibodies were found to recognize a 125 kDa protein on immunoblots of partially purified A2058 conditioned medium and to preferentially stain some breast carcinoma cells compared to normal breast using immunohistochemical techniques.

An A2058 cDNA library was prepared by purifying poly-A purified mRNA from the cells then size-selecting mRNA > 1000 bp for the preparation of cDNA. The cDNA inserts were placed into λ gt11 directionally, using the ProMega cDNA kit using standard methods well-established in the field. LE 392 cells were infected with the λ gt11 and plaques were transferred onto nitrocellulose membranes by overnight incubation at 37°C. The antibody was incubated with the membranes in blocking buffer for 2 hr at room temperature, using approximately twice the concentration of antibody which gave a strong response on Western blot analysis. Secondary antibody was goat anti-rabbit immunoglobulin, and the blot was developed colorimetrically with 4-chloro-1-naphthol.

Positive clones were confirmed by antibody competition with specific peptides but not unrelated peptides. Using this technique and multiple subclonings, we obtained a partial cDNA clone of the autotaxin gene, which we called 4C11. The 4C11 insert was removed from λ gt11 by restriction enzyme digests and subcloned into pBluescript for sequencing by standard Sanger techniques (Sanger, et al., 1977). The 4C11 clone contained bases,

EXAMPLE 7

Database analysis of the 3' terminus of the ATX gene demonstrated a novel protein. However, we have found an interesting homology that has helped to guide us in exploring its function. ATX had a 45% amino acid identity and a 57% nucleotide identity with PC-1, a marker of B cell activation found on the surface of plasma cells. Using the PC-1 protein sequence as a guide, we found that ATX peptide homologies were scattered throughout the length of the protein. The only exception was the far amino terminus of PC-1, which includes the transmembrane and intracellular domains, and which had no homologies. Knowing approximate localization of the ATX peptides along the length of ATX, we then amplified different segments of ATX by the PCR (Figure 13). These amplified segments of DNA were then subcloned into plasmids utilizing the TA Cloning kit of ProMega. The PCR amplified DNA could then be sequenced using standard Sanger sequencing techniques (Sanger, et al., 1977).

A reverse transcriptase reaction was performed using total or oligo-(dT) purified RNA from A2058 or N-tera 2D1 cells as template and an anti-sense primer from the 5' end of 4C11 (GCTCAGATAAGGAGGAAAGAG). This was followed by one or two PCR amplification of the resultant cDNA using the commercially available kit from Perkin-Elmer and following manufacturer's directions.

These PCR reactions utilized nested antisense primers from 4C11 (GAATCCGTAGGACATCTGCTT and TGTAGGCCAAACAGTTCTGAC) as well as degenerate, nested sense primers deduced from ATX peptides: ATX-101 (AAYTCIATGACARACIGTITTYGTIG and TTYGTIGGITAYGGICCIACITTYAA), ATX-103 (AAYTAYCTIACIAAYGTIGAYGAYAT and GAYGAYATIACICTIGTICCGGIAC), or ATX-224 (TGYTTYGARYTICARGARGCIGGICCC). The amplified DNA was then purified from a polyacrylamide gel using standard procedures and ligated into the pCR[™] plasmid using the TA cloning kit (Invitrogen Corporation) according to manufacturer's directions.

The 5' RACE kit was utilized to extend the 5' end of ATX cDNA using total RNA from N-tera 2D1 as template and previously obtained sequence as primer (GCTGTCTTCAAACACAGC). The 5' end of the A2058 synthesized protein was obtained by using previously obtained sequence as primer (CTGGTGGCTGTAATCCATAGC) in a reverse transcriptase reaction with total A2058 RNA as template, followed by PCR amplification utilizing the 5' end of N-tera 2D1 sequence as sense primer (CGTGAAGGCAAAGAGAACACG) and a nested antisense primer (GCTGTCTTCAAACACAGC). A2058 DNA encoding ATX is set forth in a SEQ ID NO:68 and the amino acid sequence is provided in SEQ ID NO:69.

DNA sequencing: DNA sequencing was performed using dideoxy methodology (Sanger, et al. 1977) and (³⁵S)dATP (Du Pont, New England Nuclear).

We have found one region between the 5' end of the 4C11 and the ATX peptide designated ATX-101, also referred to as the "hot spot". This region has been sequenced five times with different sequences found each time. The hot spot appears to be located within the region from approximately nucleotide 1670 to 1815. The consensus sequence is represented by amino acids position 559 through 604. Variations found include DNA sequence

that results in single and multiple amino acid insertions. One sequence had a stop codon in this region and may have represented an intron. This region has been found to be variable in forms of ATX.

EXAMPLE 8

Cloning ATX in a human teratocarcinoma cell line

The fact that ATX is present in other cancer cells was confirmed by sequence information from N-tera 2D1, a human teratocarcinoma cell line. For these cells, a prepared cDNA library in λ gt10 was amplified and the cDNA inserts were extracted. Using oligonucleotide primers based on known A2058 ATX sequence, DNA segments were amplified by PCR. The DNA segments were then subcloned into plasmids and sequenced as for A2058. We have 3104 bp DNA sequence for N-tera ATX (SEQ ID NO:66) and smaller portions thereof. This includes an open reading frame that codes for a putative protein containing 861 amino acids (SEQ ID NO:67) and smaller portions thereof. Like the A2058 ATX, the N-tera 2D1 sequence has homologies for multiple ATX peptides (Figure 15). Sequence homology between the A2058 and N-tera 2D1 cells is approximately 99%.

EXAMPLE 9

Cloning 5' end of ATX in human normal liver

The 5' end of ATX has proven difficult to obtain from either tumor cell line to date. Normal human liver mRNA was therefore amplified using the 5' RACE kit (Clontech) with known sequence from A2058 ATX as antisense primer. A DNA segment was obtained and has been sequenced. This segment codes for 979 amino acids, including an initiating methionine (SEQ ID NO:38). The putative protein sequence also includes a 20 amino acid transmembrane domain which is different from the tumor ATX's (SEQ ID NO:54), as shown in Table 7. Both tumorous forms of ATX apparently lack a transmembrane region and are instead secreted proteins.

Table 7

Nucleotide and Amino Acid Sequences Encoding Liver ATX Amino
Terminus containing the Transmembrane region

Protein Sequence (SEQ ID NO: 54)

Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Asp Ile Ser Leu Phe Thr Phe Ala
Val Gly Val Asn Ile Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala Glu Gly
Trp

DNA Sequence (SEQ ID NO: 53)

ATGGCAAGGA GGAGCTCGTT CCAGTCGTGT CAAGATATAT
CCCTGTTTAC
TTTTGCCGTT GGAGTCAATA TCTGCTTAGG ATTCACTGCA
CATCGAATTA
AGAGAGCAGA AGGATGG

EXAMPLE 10

Domains of ATX

Searches of protein databases (Pearson, et. al. 1988) confirmed that the homology between ATX and PC-1 was present throughout the length of the extracellular portion of the molecules (Buckley, et. al., 1990; Funakoshi, et. al. 1992). There is a 45% amino acid identity and a 64% similarity between the 2 protein sequences (Fig. 18). For the cDNA sequence, the identity is ~57%.

These proteins share several interesting properties and domains (Fig. 19). Both have a number of potential N-linked glycosylation sites: four for ATX (Asn54, Asn463, Asn577, Asn859) and nine for PC-1. Both have adjacent somatomedin B domains near the amino end of the extracellular domain. This somatomedin B domain is a cysteine-rich region containing 3 presumed cystine cross-

linkages. ATX has 33 Cys residues and PC-1 has 37; 30 of these Cys residues are identical in placement. Both proteins also contain the loop region of an EF hand (Buckley, et. al. 1990; Kretsinger, 1987). In addition, both proteins have a transmembrane/signal peptide region with a short intracellular peptide, common in ectoenzymes (Maroux, 1987). However, the amino acid identity between ATX and PC-1 in the intracellular and transmembrane regions is only 11%.

Finally, both proteins have a region homologous to the bovine intestinal phosphodiesterase enzymatic domain with conservation of the threonine that is thought to act as the intermediate phosphate binding site (Culp, et al. 1985). PC-1 has been demonstrated to have phosphodiesterase type I, nucleotide pyrophosphatase, and threonine-specific kinase enzymatic activities (Rebbe, et al. 1991; Oda, et al. 1991). In order to test whether purified ATX had type I phosphodiesterase activity, samples were incubated with p-nitrophenyl thymidine-5'-monophosphate at pH 8.9 for 30 min. Samples were assayed in a 100 μ l volume containing 50 mM Tris-HCl, pH 8.9 and 5 mM p-nitrophenyl thymidine-5'-monophosphate. After a 30 minute incubation at 37 °C the reactions were terminated by addition of 900 μ l 0.1 N NaOH and the amount of product formed was determined by reading the absorbance at 410 nm. ATX was found to hydrolyze the p-nitrophenyl thymidine-5'-monophosphate (Razzell, 1963) at a rate of 10 pmol/ng/min, a reaction rate similar to that reported for PC-1 (Oda, et al. 1993).

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All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the present invention and appended claims.

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CLAIMS:

1. A DNA segment coding for a polypeptide comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

2. The DNA segment according to claim 1, wherein said DNA segment encodes the amino acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38 through SEQ ID NO:52, SEQ ID NO:66 and SEQ ID NO:69.

3. An isolated polypeptide comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

4. The polypeptide according to claim 3, wherein said amino acid sequence comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO:36, and SEQ ID NO:38 through SEQ ID NO:52, SEQ ID NO:67 and SEQ ID NO:69.

5. An isolated polypeptide bound to a solid support, comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

6. The polypeptide according to claim 5, wherein said polypeptide comprises the amino acid sequence selected from the group consisting of the SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO: 36, SEQ ID NO:38 through SEQ ID NO:52., SEQ ID NO:67 and SEQ ID NO:69.

7. A recombinant DNA molecule comprising a vector and the DNA segment according to claim 1.

8. A cell that contains the recombinant DNA molecule according to claim 7.

9. An antibody having binding affinity for autotaxin, or binding fragment thereof.

10. A method of producing a recombinant autotaxin polypeptide said method comprising:

culturing a cell containing the recombinant DNA molecule of claim 7 under conditions such that the DNA segment is expressed, producing said polypeptide; and
isolating said polypeptide.

11. A method of purifying the autotaxin peptide of claim 3, comprising the steps of:

i) collecting and concentrating supernatant from cultured A2058 human melanoma cells whereby a first preparation of said peptide is produced;

ii) salt fractionating said first preparation to produce a second peptide preparation;

iii) isolating said peptide from said second preparation so that said peptide is obtained in substantially pure form.

12. The method of claim 11, wherein said isolating step is effected by column chromatography.

13. An isolated DNA encoding an autotaxin protein or fragment thereof wherein said DNA includes a nucleic acid sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:37 and SEQ ID NO:38.

14. The DNA segment according to claim 1, wherein said DNA fragment comprises any one of the SEQ ID NO:12 through SEQ ID NO:25, or SEQ ID NO:39 through SEQ ID NO:52.

15. The DNA segment according to claim 13 wherein said DNA segment comprises any one of the SEQ ID NO:12 through SEQ ID NO:25.

16. An isolated polypeptide comprising an amino acid sequence corresponding to autotaxin.

17. A polypeptide bound to a solid support and comprising an amino acid sequence corresponding to autotaxin.

18. A recombinant autotaxin polypeptide

according to claim 3.

19. An isolated polypeptide according to claim 3 having cell motility activity.

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ABSTRACT OF THE DISCLOSURE

The present invention relates, in general, to autotaxin. In particular, the present invention relates to a DNA segment encoding autotaxin; recombinant DNA molecules containing the DNA segment; cells containing the recombinant DNA molecule; a method of producing autotaxin; antibodies to autotaxin; and identification of functional domains in autotaxin.

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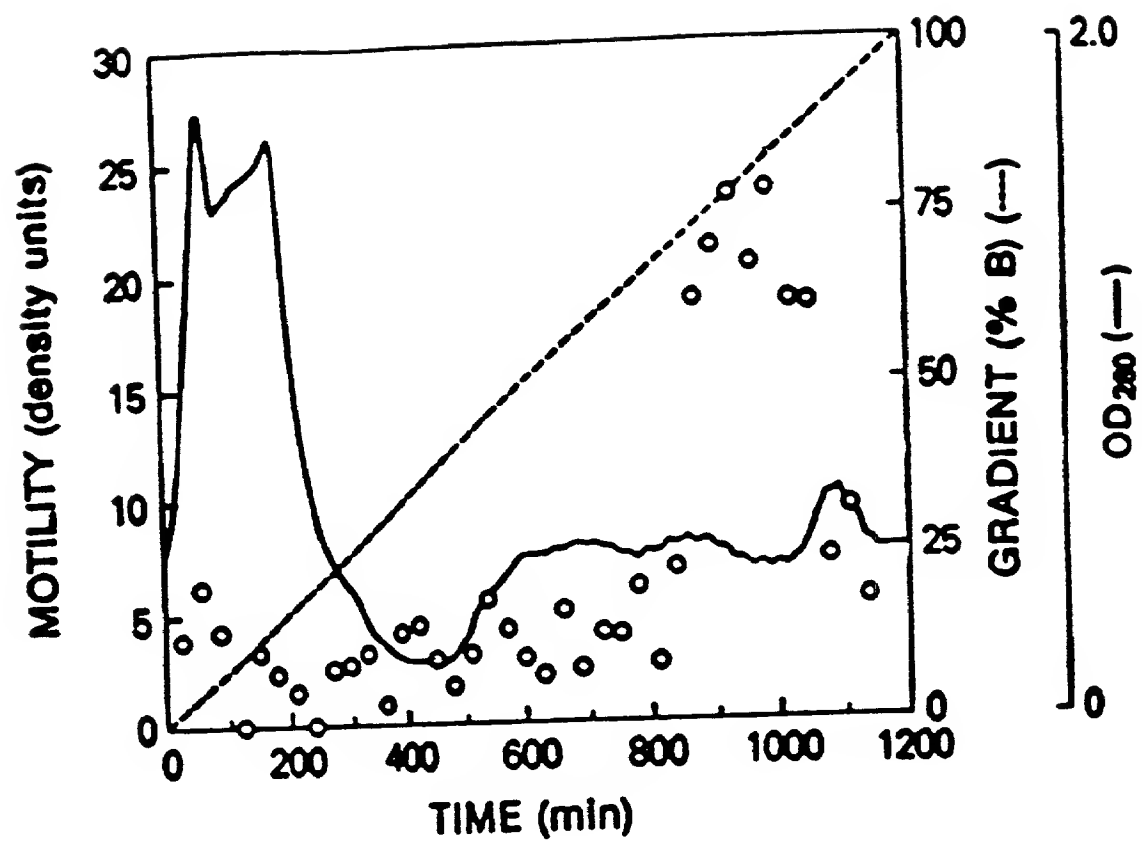


FIGURE 1

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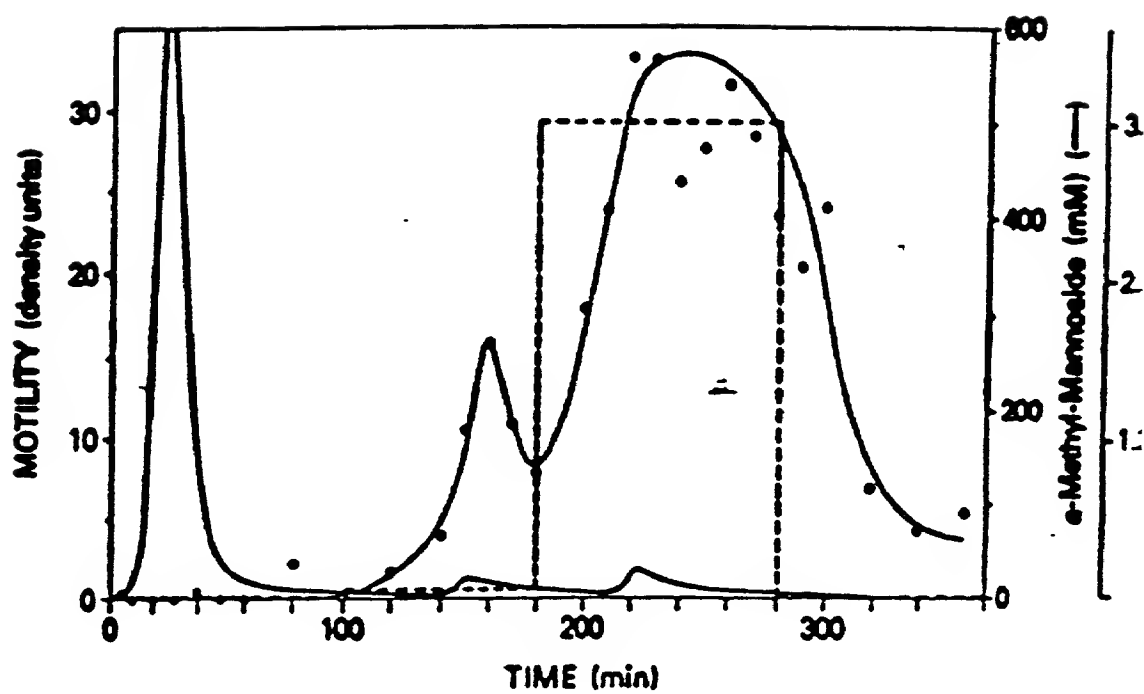


FIGURE 2

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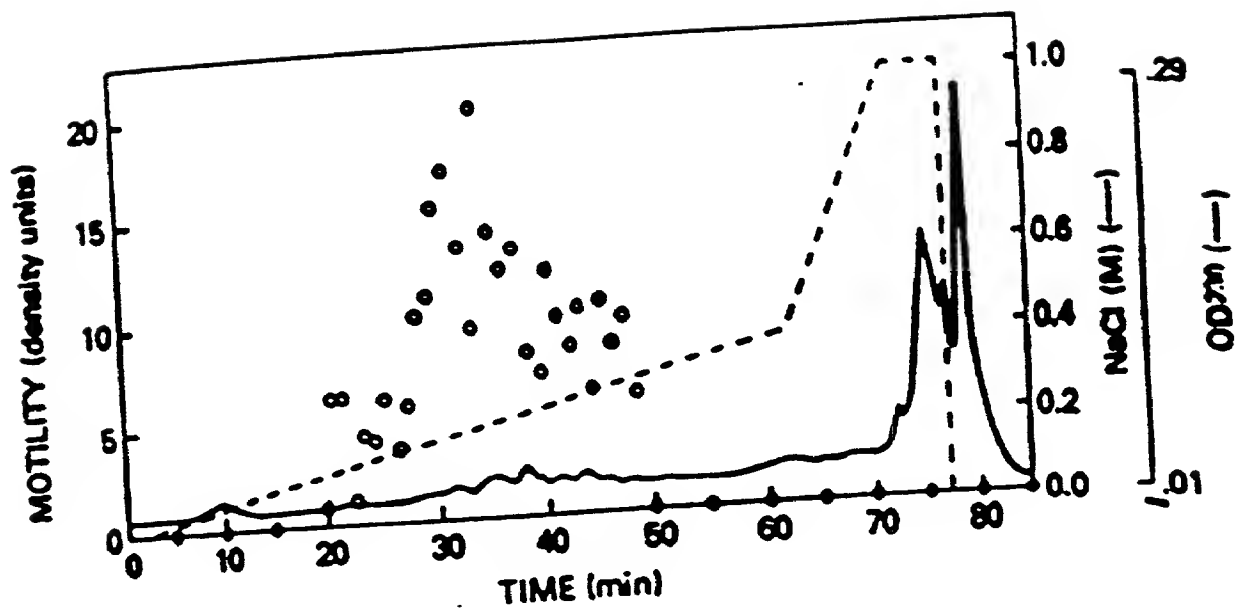


FIGURE 3

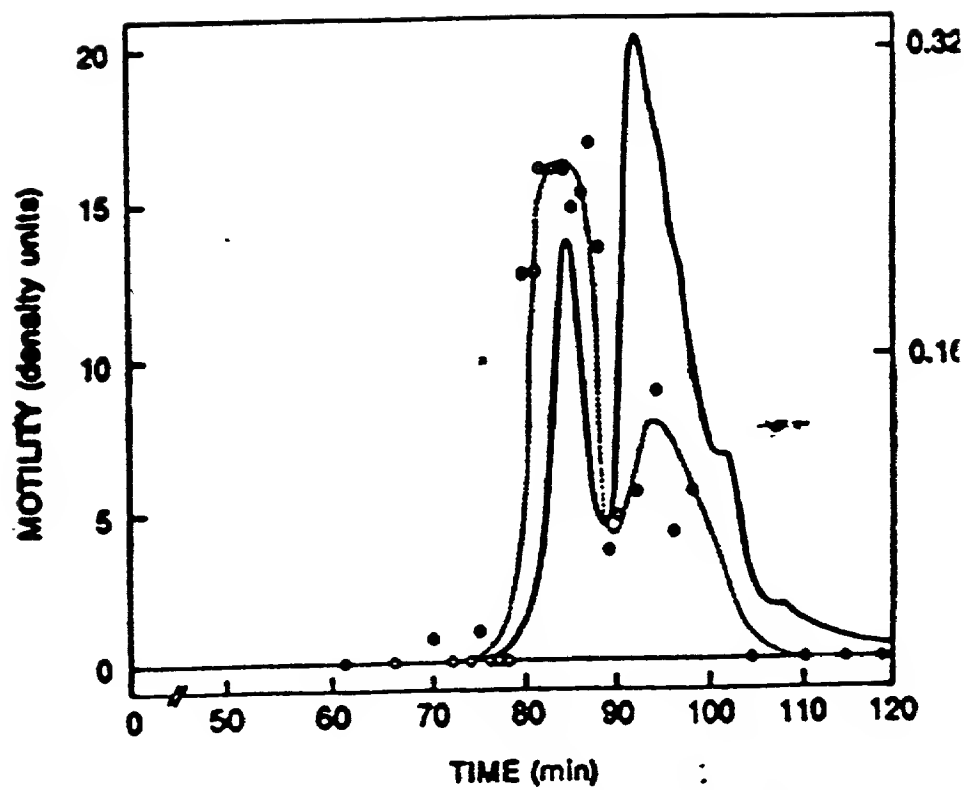


FIGURE 4

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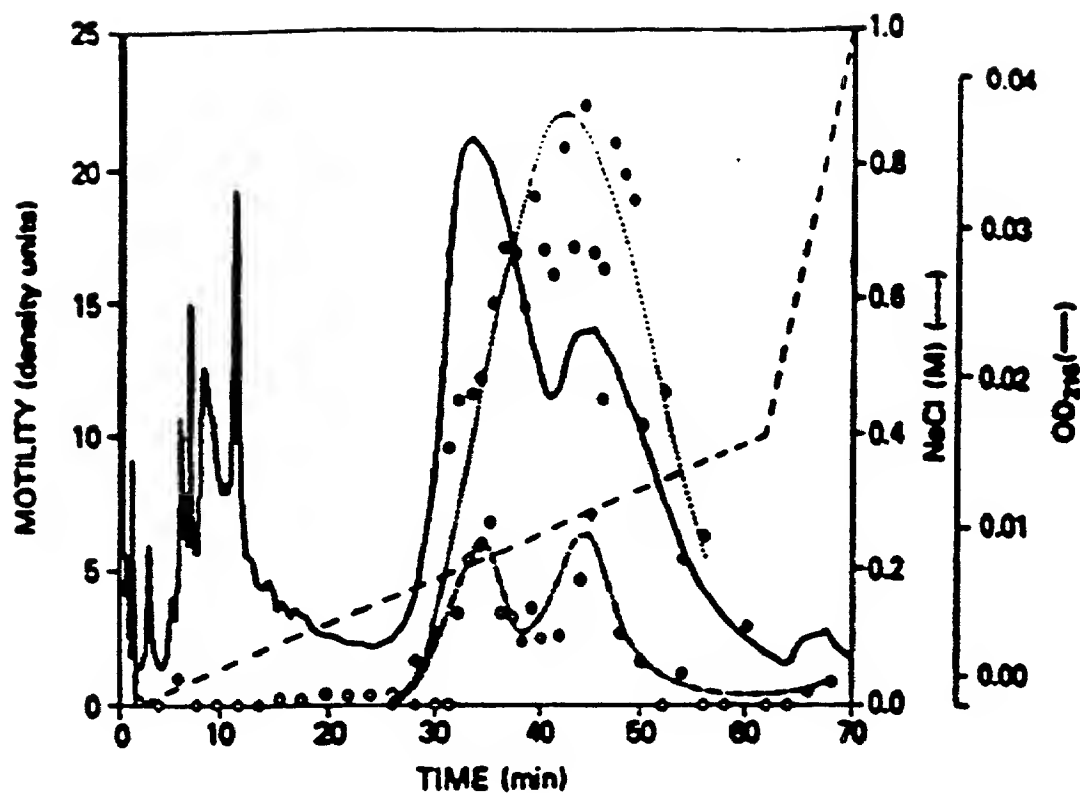


FIGURE 5

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09462234 044700



FIGURE 6

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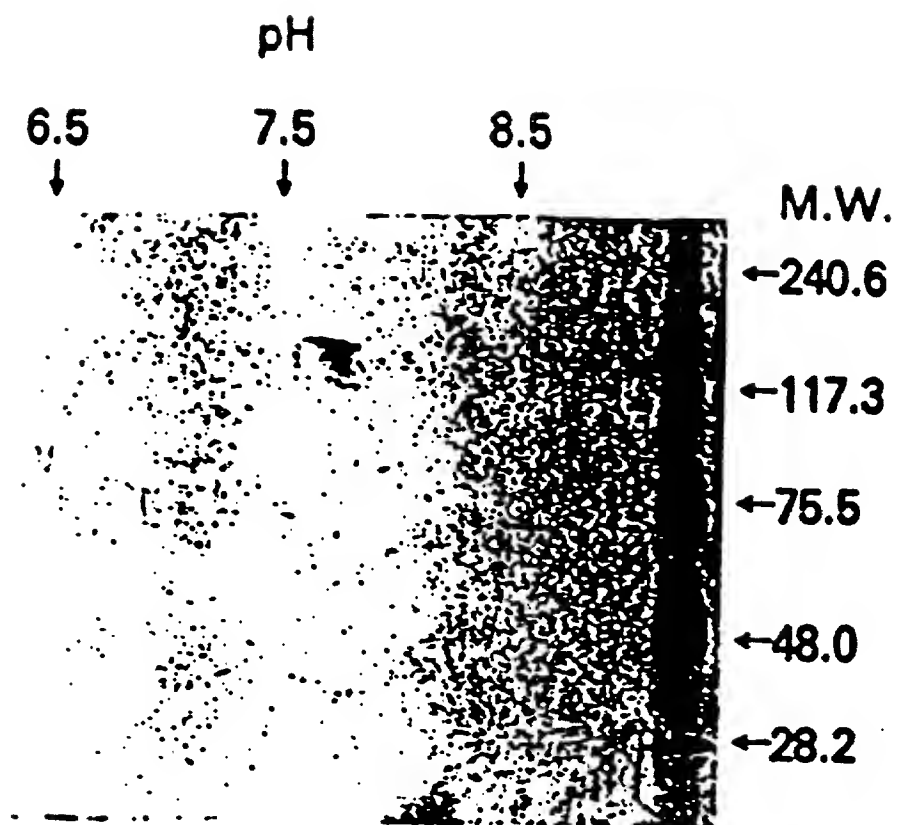


FIGURE 7

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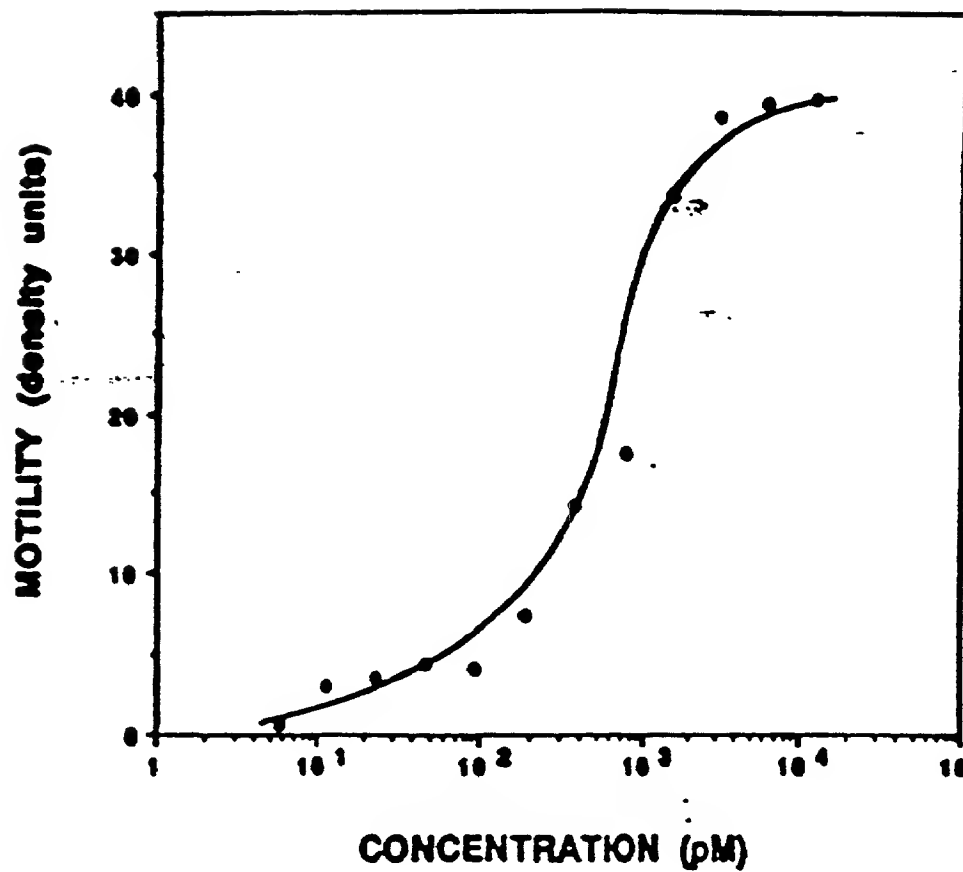


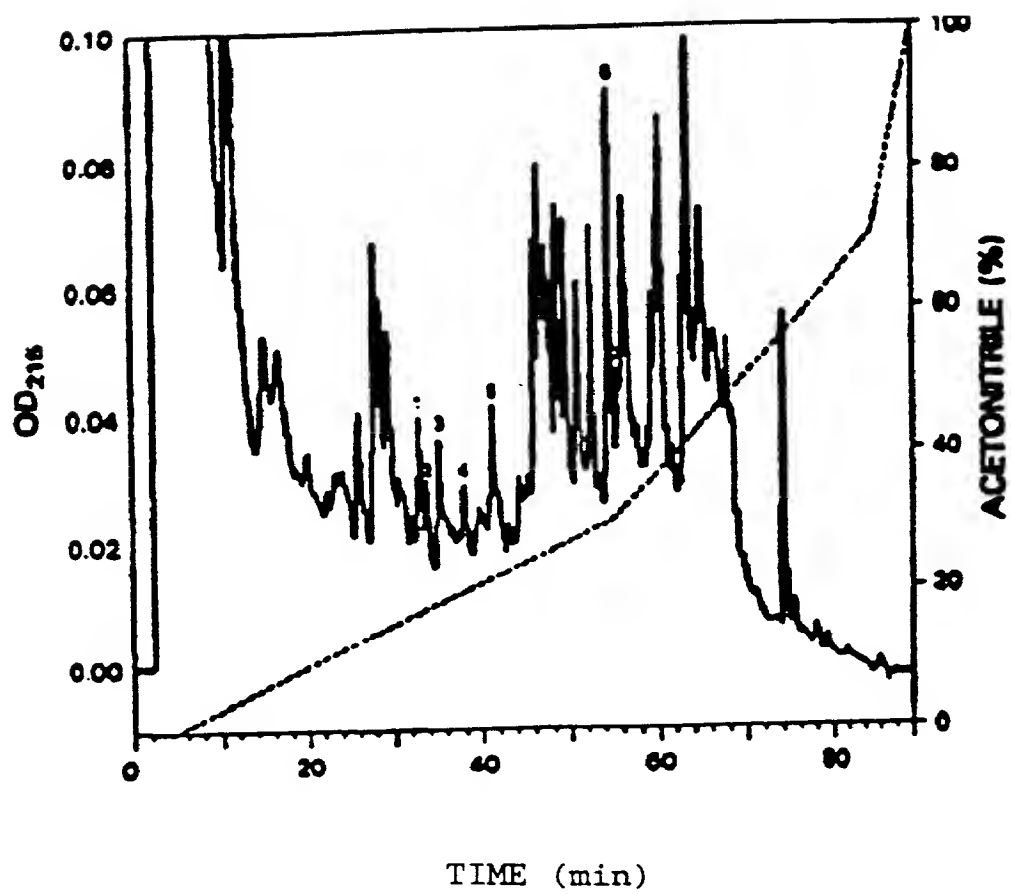
FIGURE 8

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FIG. 10

		Upper Wells		
		0	0.01%	0.1%
Lower Wells	0	4.8 ± 0.3	13.7 ± 0.8	33.8 ± 1.0
	0.01%	45.4 ± 4.0	39.3 ± 2.0	36.0 ± 1.4
	0.1%	75.6 ± 1.0	66.3 ± 2.1	41.9 ± 2.4

FIG. 11



**cDNA Cloning
of ATX
(4 C11 clone)**

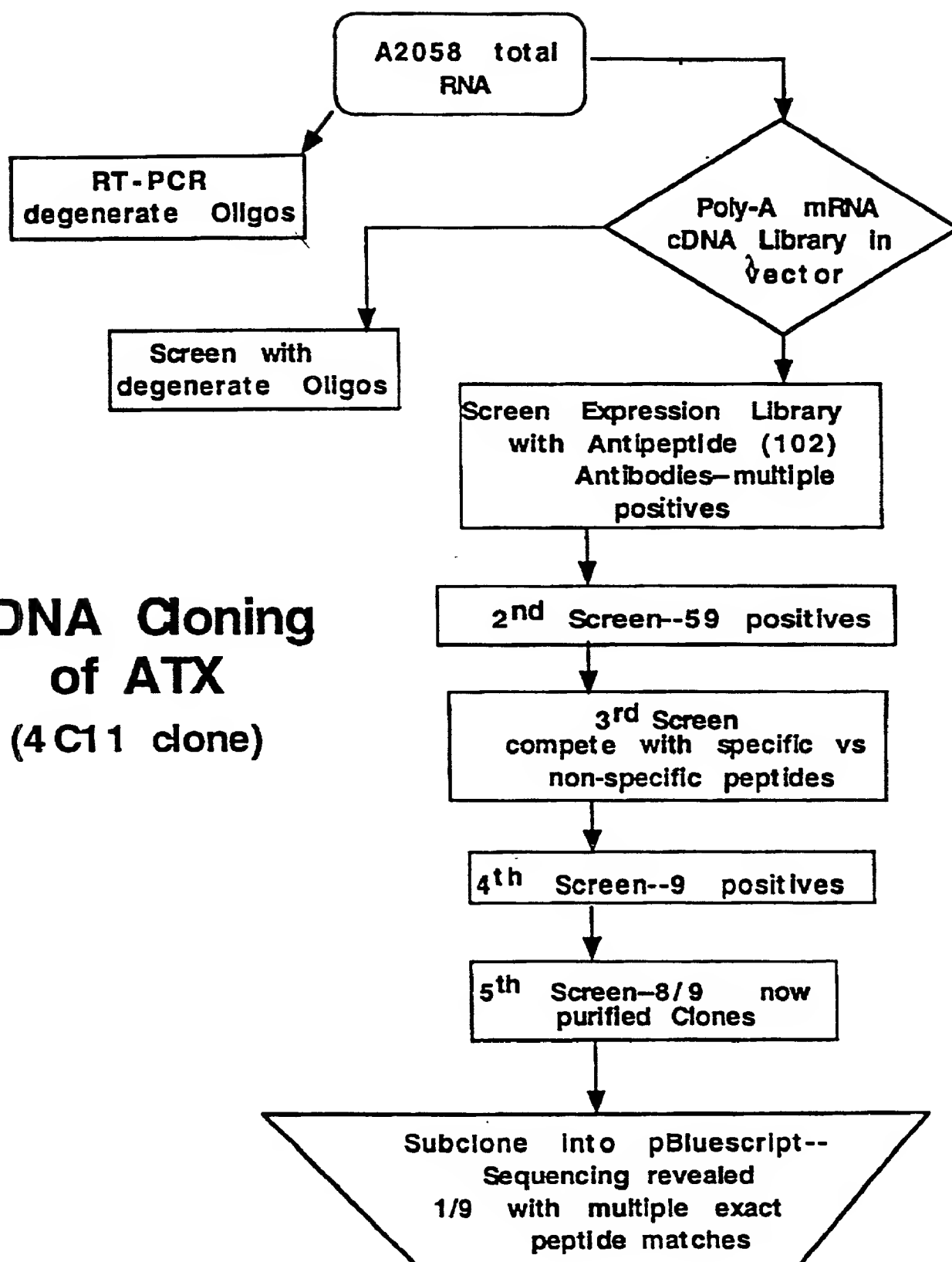


FIGURE 12

AUTOTAXIN GENE

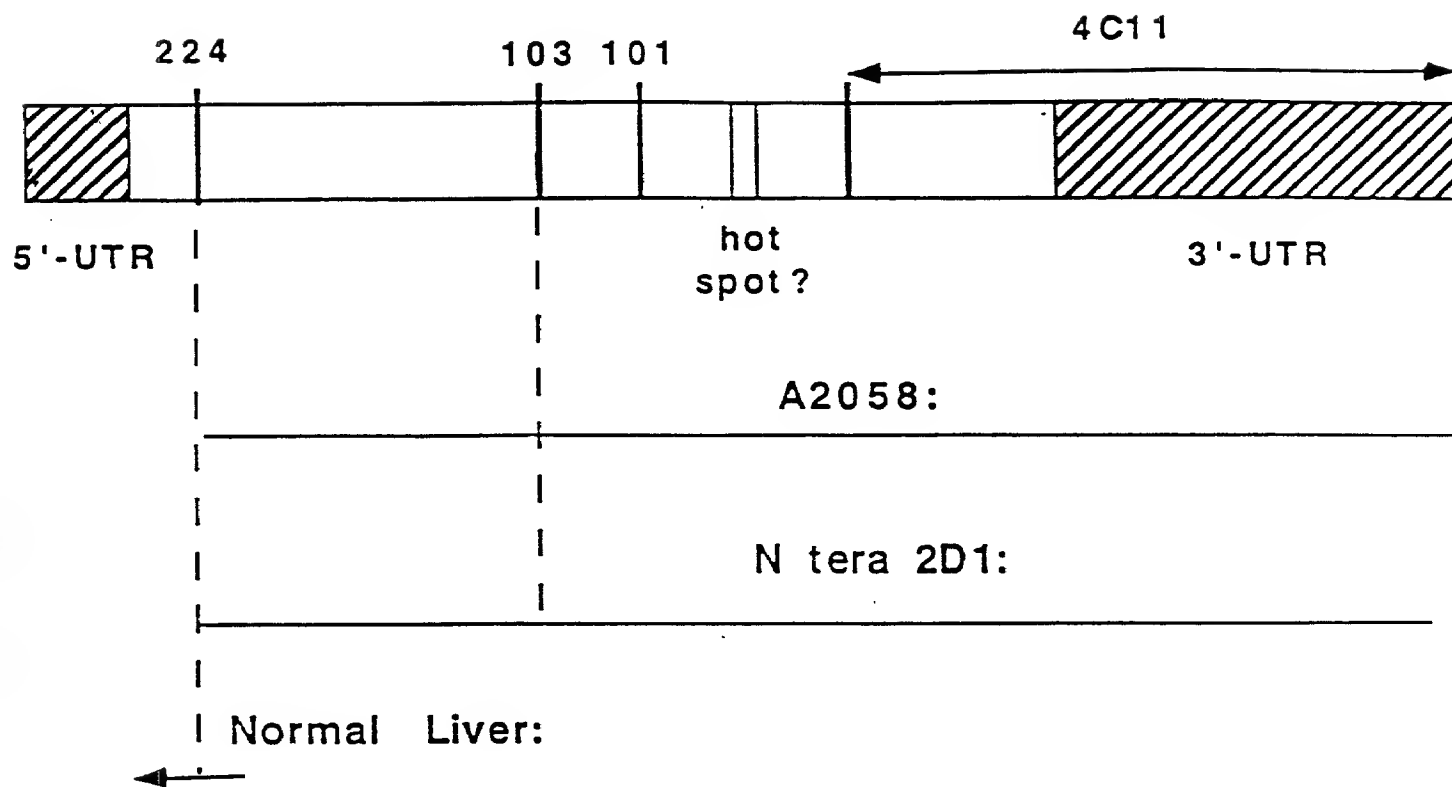


FIGURE 13

[illegible]

Match-up of ATX peptides with putative N-tera 2D1 protein sequence

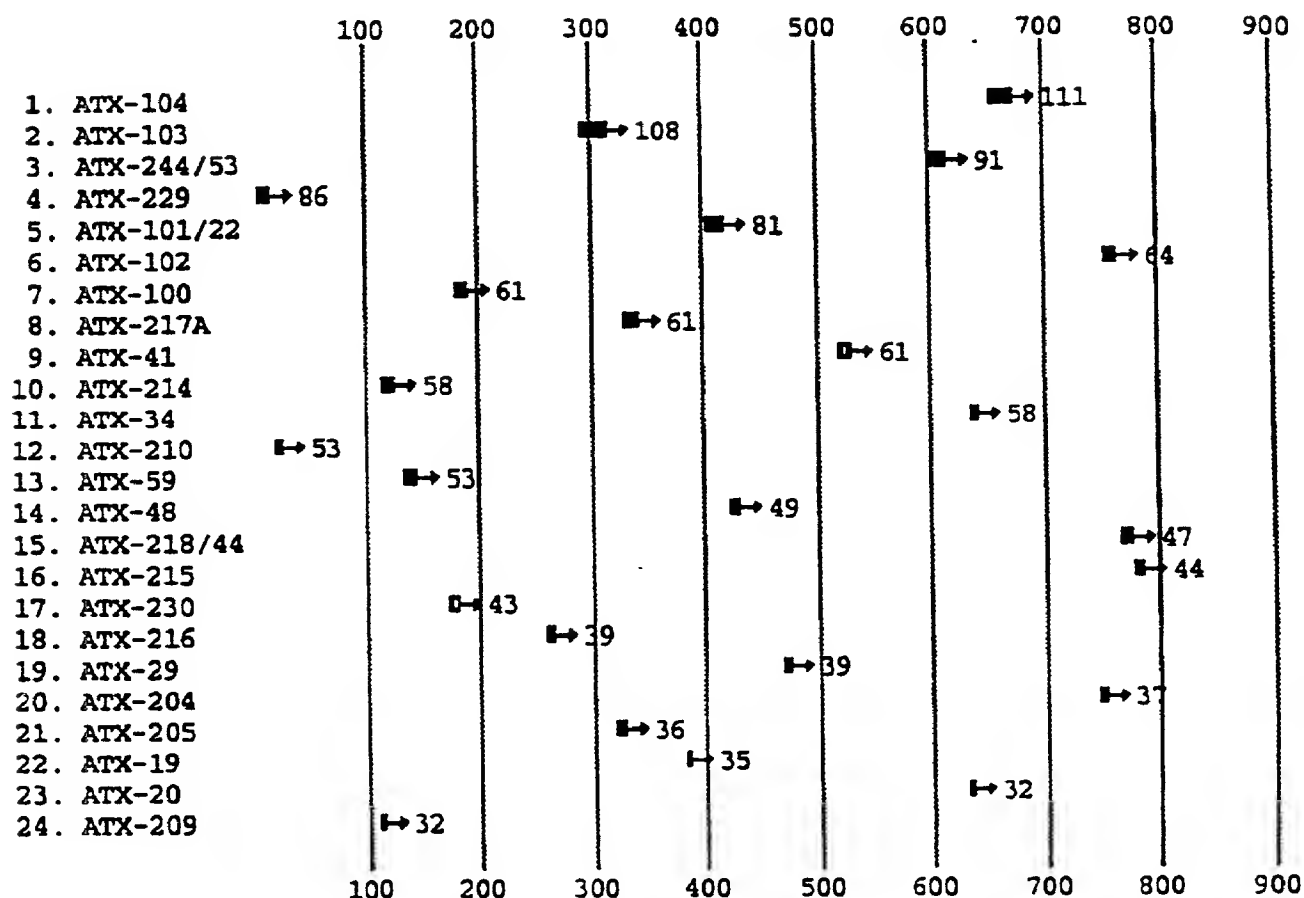
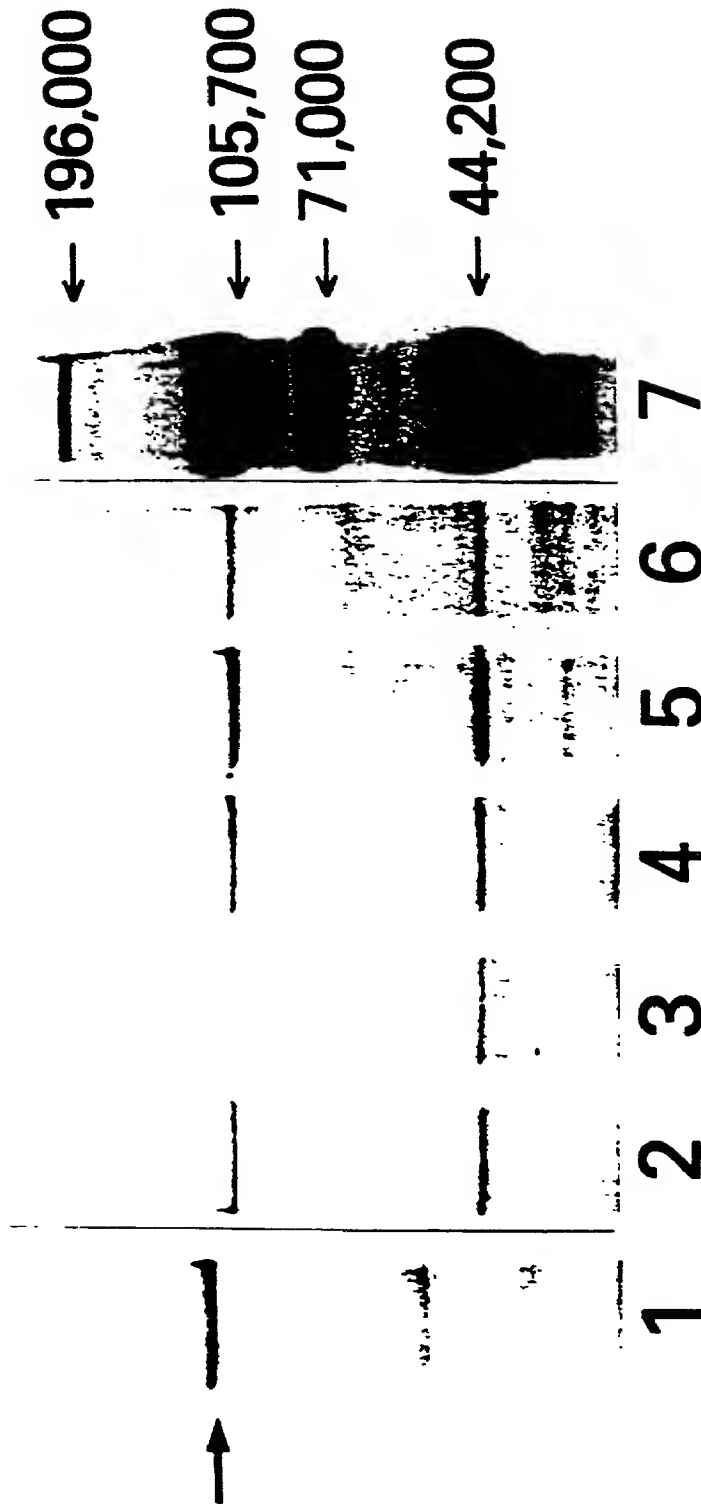


FIGURE 15

FIG. 16



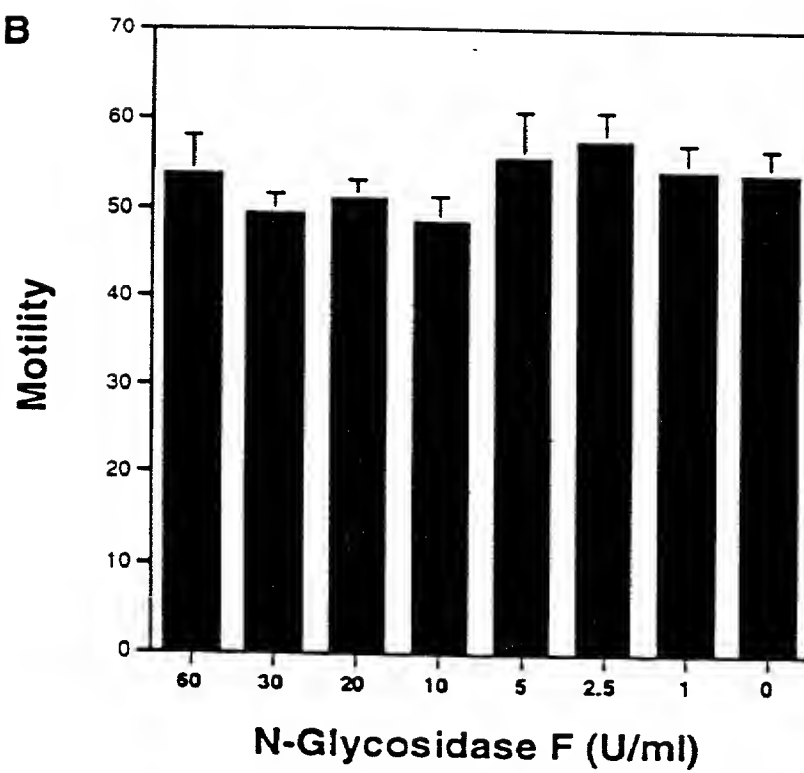
002310-1E9E8H60

FIG. 17

A

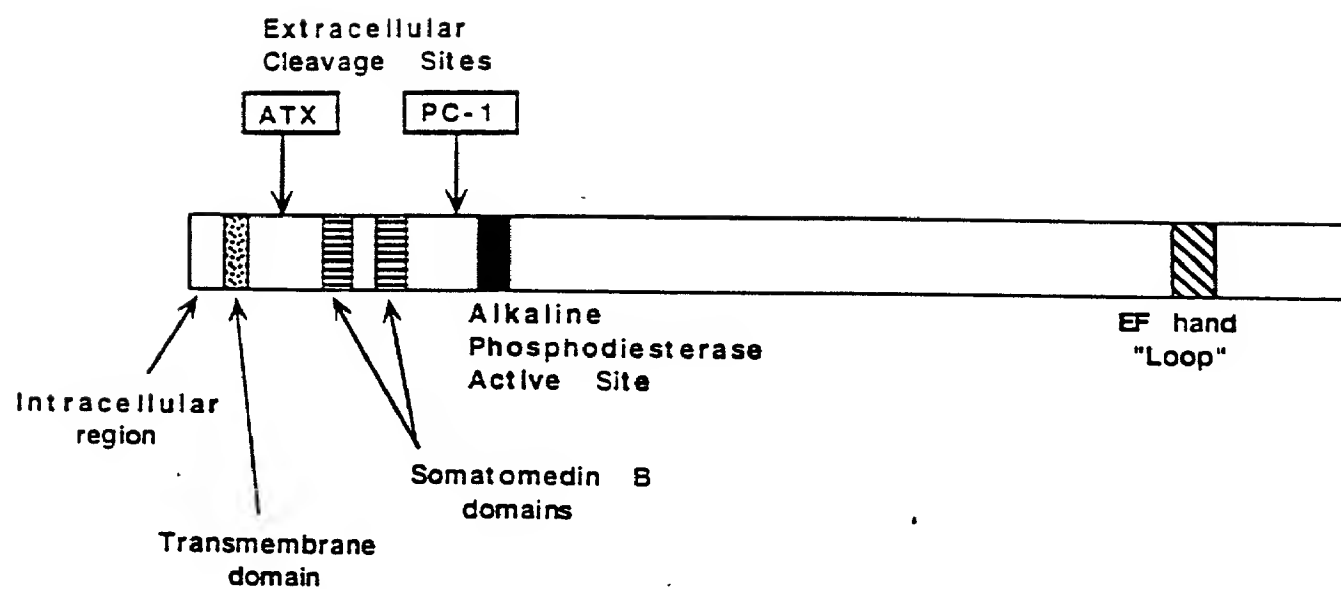


B



hATX	MARRSSFQSCQIIISLFTFAVGVSIICLGFATIRIKRAEGWEEGPTVLSDSPWNTISGCKGRCFELQEAQPPDCRCDNLCKSYTSCCII	90
hPC1	MDVGEEPLEKAARARTAKDPNTYKVI.SLVL.SVCVLTTI.....GCIFG...LKPSCAKEVK.SCKGRCF...ERTFGICKDAACVELJHCCIDA	81
hATX	DELCLKTARGWECTKDRCGEVRNEENACHCEDCLARGDCCTNYQVCKGESHMWDDDCETKAACECPAGFVRPPLIIFSVDGFRAASYMKKSGKVNPHIE	190
hPC1	QETCIEPEIHWTCNKFRCGEKRI.TRSICACSDACKJGDCCTINYSSVCQGEKSWREPCESINEPQCPACFTTPTTJ.FSLDGFRAEYIJTWGGLH.PVIF	184
hATX	KLKRCGTHSPYMRPVYPTKTFPNLYTLATGLYPESHGIVGNSMYDPVDFATFHLRGREKENHWRMGQPLWITATKQGVKAGTFEWS.....	272
hPC1	KLKCKGTYTKNMRPVYPTKTFPNHYSIWTLGYPESHGIIIDNKMYDPKMNASFSLKSEKENPEWYKGEPIWVTAKYQGLKSGTFWPGSDVEINGIFPDI	284
hATXWIFIHERRILTLRWLTL.PDIHERPSVYAFYSEQPIHFSGHKYGPPGPEESSYSGSPFPAKRPKRKVAPKKRKRKIIIRNDIHYAAET	372
hPC1	YKMYNGSVPFEEILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSGYGPVSSE.....	336
hATX	RQDKMTNPLREIDKIVQQLMDGLKQLKLRRCVNVIFVGDHGMEDVTCDRTEFLSNLYTNVDDITLVPGLGRIR.SKFSNN.AKYDPKAIIANLTCKKPD	470
hPC1VIKALQRVDMVGMMLDGLKELNLHJRCNLILISDIGMEQSCKKYIYNKYLGDVKNIKVIYGPAAKLRPSDVPDKYYSFNIEGIARNL.SCKEPI	432
hATX	QHFKPYLKQILPKRLIYANNRRIEDIIILLVERRWIVARKPLDVYKPSGKCFQGDHGHKVNMQTFVGYGPTFKYKTKVPPEHIELYIIVMCDIJG	570
hPC1	QHFKPYLKHLFPKRLHFAKSDRIEPLTFYLDPOQWQALNPSE..RKYCGSGF....HGSNDVFSNMQALFVGYGPGFKHIGIEADTFENIEVYNI.MCDILH	526
hATX	LKPAPNNGTHGSLNHLRLTNTFRPTMPEEVTRPNYPGIMYLSQDFDLGCTCDDKVEPKNKLD.ELNKRLIITKGSTEERHLLYGRPAVLYRTR.YDILYHT	668
hPC1	LTPAPNNGTHGSLNHLKPNVYTPKHPEV.HPLVQCPTFRNPRDNLGCSCNPSILPIEDFQTQFNLTVAEEKI IKHETLPYGRPRVLQKENTICLLSQH	625
hATX	DFESGYSEIFLMLLWTSYTVSKQAEVSSVPDHLTSCVRPDVRVSPFSQNCLAYKNDKQMSYGLFPPYLSSSPEAKY.DAFLVTNMVPMYPAFKRVVNY	767
hPC1	QFMSGYSQDILMPLWTSYTVDRNDSFS...TEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPQNLKNSSGIYSEALLTTNIVPMYQSFQVIWKY	723
hATX	FQRLVLKKYASERNGVNVISGPIFDYDGLHDTEDIKQ...YVEGSSIPVPHYYSIITSCLDFTQPADKCDGPLSVSSFILPHRPDNEESCNSSEDE	875
hPC1	FHDTLLRKYAEERNGVNVSGPVDFDYDGRCDSEIENLRQKRRVIRNQEILIPHTFFIVLTSCKOTSQTPHLEN.LDTLAFILPHRTDNSESCVIIGKHD	822
hATX	SKWVEELMKMHTARVRDIEHLTSLDFFRKTSRSYPEILTLKTYLHTYESEI	915
hPC1	SSWVEELMLHRARITDVEHITGLSFYQQRKEPVSDILKLKTHLPTFSQED	873

FIG. 19



COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, the information given herein is true, that I believe I am the original, first and sole (if only one name is listed below) or an or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: AUTOTAXIN: MOTILITY STIMULATING PROTEIN USEFUL IN CANCER
DIAGNOSIS AND THERAPY

which is described in: ☐ PCT International Application No. _____ filed _____
☐ the attached application or ☒ the specification in application Serial No. 08/346,455 filed November 28, 1994
(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known to me which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56 (a).

I hereby claim foreign priority benefits under Title 35 United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign applications(s) for patent or inventor's certificate or any PCT international applications(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

COUNTRY	APPLICATION	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC § 119
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) or PCT International application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

Application Serial No.	Filing Date	Status: patented, pending, abandoned
07/822,043	January 17, 1992	Pending
08/249,182	May 25, 1994	Pending

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

James C. Haight, Reg. No. 25,588; Gloria Richmond, Reg. No. 30,416; Robert Benson, Reg. No. 33,612; Jack Spiegel, Reg. No. 34,477; Laurence J. Hyman, Reg. No. 35,551; Denise C. Bernstein, Reg. No. 35,787; Susan S. Rucker, Reg. No. 35,762; David R. Sadowski, Reg. No. 32,808 and Ann S. Hobbs, Reg. No. 36,830 and Arthur J. Cohn, Reg. No. 37,800 all of the Office of Technology Transfer, National Institutes of Health, BOX OTT, Bethesda, MD 20892.

I further direct that all correspondence concerning this application be directed to:

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National Institutes of Health
Box OTT
Bethesda, MD 20892
Telephone: (301) 496-7056
Fax: (301) 402-0220

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature: *Mary Stracke* Date: 1/31/95

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Inventor's signature: *Lance Liotta* Date: 1-31-95

Country of Citizenship: United States of America

Residence: 9027 Mistwood Drive, Potomac, Maryland, 20854, U.S.A.

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Inventor's signature: *Elliott Schiffmann* Date: 1/31/95

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00463331 041760

Docket No. 2026-4149US2

Full Name of fourth inventor: Jerry Krutzch

Inventor's signature: _____ Date: _____

Country of Citizenship: United States of America

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Post Office Address: 9704 De Paul Drive, Bethesda, Maryland 20817, U.S.A.

Full Name of first fifth inventor: Jun Murata

Inventor's signature: *Jun Murata* Date: 2/01/95

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Docket No. 2026-4149US2

Full Name of fourth inventor: Henry Krutzsch

Inventor's signature: Henry Krutzsch Date: 1/31/95

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Post Office Address: 9704 De Paul Drive, Bethesda, Maryland 20817, U.S.A.

Full Name of first fifth inventor: Jun Murata

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
Stracke, et al.) Art Unit: 1806
Serial Number: 08/346,455)
Filed: November 28, 1994) Examiner: Loring, S.
For: Autotaxin: Motility Stimulating Protein Useful)
in Cancer Diagnosis and Therapy)

Associate Power of Attorney

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

The undersigned attorney of record, pursuant to the provisions of 37 CFR §1.33 and § 1.34 M.P.E.P. §402.2, hereby appoints the following as associate attorneys, with full powers to prosecute this application:

Jerome G. Lee, Reg. No. 16,967; John D. Foley, Reg. No. 16,836; John A. Diaz, Reg. No. 19,550; Thomas P. Dowling, Reg. No. 19,221; Eugene Moroz, Reg. No. 25,237; William S. Feiler, Reg. No. 26,728; Israel Blum, Reg. No. 26,710; Mary Morry, Reg. No. 34,398; Maria C. H. Lin, Reg. No. 29,323; Eugene Rzucidlo, Reg. No. 31,900; John C. Vassil, Reg. No. 19,098; Leslie A. Serunian, Reg. No. 35,353; Kathryn M. Brown, Reg. No. 34,556; Dorothy R. Auth, Reg. No. 36,434 and Richard W. Bork, Reg. No. 36,459;


all of the law firm of MORGAN & FINNEGAN, 345 Park Avenue, New York, NY 10154.

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Respectfully submitted,


Susan S. Rucker
Attorney for Applicant(s)
Reg. No. 35,762

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- (i) APPLICANT: UNITED STATES OF AMERICA; DEPT.
OF HEALTH AND HUMAN SERVICES
- (ii) TITLE OF INVENTION: MOTILITY STIMULATING
PROTEIN USEFUL IN CANCER DIAGNOSIS AND
THERAPY
- (iii) NUMBER OF SEQUENCES: 69
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 - (B) STREET: 345 PARK AVENUE
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 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 10154
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 - (A) MEDIUM TYPE: Floppy Disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: WordPerfect 5.1
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA
 - (A) APPLICATION NUMBER: 08/346,455
 - (B) FILING DATE: 28-NOV-1994
- (vii) PRIOR APPLICATION DATA
 - (A) APPLICATION NUMBER: 08/249,182
 - (B) FILING DATE: 25-MAY-1994
- (vii) PRIOR APPLICATION DATA
 - (A) APPLICATION NUMBER: 07/822,043
 - (B) FILING DATE: 17-JAN-1992
- (viii) ATTORNEY/AGENT INFORMATION:
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 - (C) DOCKET NUMBER: 2026-4149US3
- (ix) TELECOMMUNICATION INFORMATION:
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00279-1280460

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Trp His Val Ala Arg
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Pro Leu Asp Val Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Tyr Pro Ala Phe Lys
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gln Ala Glu Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Pro Glu Glu Val Thr Arg Pro Asn Tyr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Tyr Asp Val Pro Trp Asn Glu Thr Ile
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Val Pro Pro Phe Glu Asn Ile Glu Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Gly Gln Pro Leu Trp Ile Thr Ala Thr Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Val Asn Ser Met Gln Thr Val Phe Val Gly Tyr Gly
1 5 10

Pro Thr Phe Lys
15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Ile Glu His Leu Thr Ser Leu Asp Phe Phe Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Thr Glu Phe Leu Ser Asn Tyr Leu Thr Asn Val Asp
1 5 10

Asp Ile Thr Leu Val Pro Glu Thr Leu Gly Arg
15 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTGGCAGCN ACRTGCCA

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGCAYGTNG CTGCCAAC

18

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTGAAGGCA GGGTA

15

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TAYCCTGCNT TYAAG

15

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTNACYTCY TCAGG

15

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15
 - (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCTGARGARG TNACC

15

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

NGTNGCRTCR AATGGCACRT C

21

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAYGTGCCAT TYGAYGCNAC N

21

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTTDATRTTS TCRAATGGGG G

21

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCCCCATTTG AGAACATCAA C

21

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTTNGTNGCN GTDATCCANA RGGGYTGGCC GCC

33

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGCGGCCARC CCYTNTGGAT HACNGCNACN AAG

33

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTTRAAGGTG GGGCCRTAGC CCACRAAGAC TGTYTG CAT

39

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATGCARACAG TCTTYGTGGG CTAYGGCCCC ACCTTYAAR

39

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gln Tyr Leu His Gln Tyr Gly Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Val Leu Asn Tyr Phe
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Tyr Leu Asn Ala Thr
1 5

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

His Leu Leu Tyr Gly Arg Pro Ala Val Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Tyr Pro Glu Ile Leu Thr Pro Ala Asp Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Xaa Tyr Gly Phe Leu Phe Pro Pro Tyr Leu Ser Ser
1 5 10
Ser Pro

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Thr Phe Pro Asn Leu Tyr Thr Phe Ala Thr Gly Leu
1 5 10
Tyr

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Val Asn Val Ile Ser Gly Pro Ile Asp Asp Tyr Asp
1 5 10
Tyr Asp Gly Leu His Asp Thr Glu Asp Lys
15 20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 829
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Human
- (B) STRAIN:
- (C) INDIVIDUAL ISOLATE:
- (D) DEVELOPMENTAL STAGE:
- (E) HAPLOTYPE:
- (F) TISSUE TYPE:
- (G) CELL TYPE: Melanoma
- (H) CELL LINE: A2058
- (I) ORGANELLE:

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Putative protein
sequence of A2058 Autotaxin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Cys	His	Asp	Phe	Asp	Glu	Leu	Cys	Leu	Lys	Thr	Ala	
1				5					10			
Arg	Gly	Trp	Glu	Cys	Thr	Lys	Asp	Arg	Cys	Gly	Glu	
		15					20					
Val	Arg	Asn	Glu	Glu	Asn	Ala	Cys	His	Cys	Ser	Glu	
25					30					35		
Asp	Cys	Leu	Ala	Arg	Gly	Asp	Cys	Cys	Thr	Asn	Tyr	
			40					45				
Gln	Val	Val	Cys	Lys	Gly	Glu	Ser	His	Trp	Val	Asp	
	50					55					60	
Asp	Asp	Cys	Glu	Glu	Ile	Lys	Ala	Ala	Glu	Cys	Pro	
				65					70			
Ala	Gly	Phe	Val	Arg	Pro	Pro	Leu	Ile	Ile	Phe	Ser	
		75					80					
Val	Asp	Gly	Phe	Arg	Ala	Ser	Tyr	Met	Lys	Lys	Gly	
85					90					95		
Ser	Lys	Val	Met	Pro	Asn	Ile	Glu	Lys	Leu	Arg	Ser	
			100					105				
Cys	Gly	Thr	His	Ser	Pro	Tyr	Met	Arg	Pro	Val	Tyr	
	110					115					120	
Pro	Thr	Lys	Thr	Phe	Pro	Asn	Leu	Tyr	Thr	Leu	Ala	
				125					130			
Thr	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Val	Gly	
		135					140					
Asn	Ser	Met	Tyr	Asp	Pro	Val	Phe	Asp	Ala	Thr	Phe	
145					150					155		
His	Leu	Arg	Gly	Arg	Glu	Lys	Phe	Asn	His	Arg	Trp	
			160					165				
Trp	Gly	Gly	Gln	Pro	Leu	Trp	Ile	Thr	Ala	Thr	Lys	
	170					175					180	
Gln	Gly	Val	Lys	Ala	Gly	Thr	Phe	Phe	Trp	Ser	Val	
				185					190			
Val	Ile	Pro	His	Glu	Arg	Arg	Ile	Leu	Thr	Ile	Leu	
		195					200					
Arg	Trp	Leu	Thr	Leu	Pro	Asp	His	Glu	Arg	Pro	Ser	
205					210					215		
Val	Tyr	Ala	Phe	Tyr	Ser	Glu	Gln	Pro	Asp	Phe	Ser	
			220					225				
Gly	His	Lys	Tyr	Gly	Pro	Phe	Gly	Pro	Glu	Glu	Ser	
	230					235					240	
Ser	Tyr	Gly	Ser	Pro	Phe	Thr	Pro	Ala	Lys	Arg	Pro	
				245					250			
Lys	Arg	Lys	Val	Ala	Pro	Lys	Arg	Arg	Gln	Glu	Arg	
		255					260					
Pro	Val	Ala	Pro	Pro	Lys	Lys	Arg	Arg	Arg	Lys	Ile	
265					270					275		
His	Arg	Met	Asp	His	Tyr	Ala	Ala	Glu	Thr	Arg	Gln	
			280						285			

Asp	Lys	Met	Thr	Asn	Pro	Leu	Arg	Glu	Ile	Asp	Lys
290						295					300
Ile	Val	Gly	Gln	Leu	Met	Asp	Gly	Leu	Lys	Gln	Leu
				305					310		
Lys	Leu	Arg	Arg	Cys	Val	Asn	Val	Ile	Phe	Val	Gly
		315				320					
Asp	His	Gly	Met	Glu	Asp	Val	Thr	Cys	Asp	Arg	Thr
325					330					335	
Glu	Phe	Leu	Ser	Asn	Tyr	Leu	Thr	Asn	Val	Asp	Asp
			340					345			
Ile	Thr	Leu	Val	Pro	Gly	Thr	Leu	Gly	Arg	Ile	Arg
350						355					360
Ser	Lys	Phe	Ser	Asn	Asn	Ala	Lys	Tyr	Asp	Pro	Lys
				365					370		
Ala	Ile	Ile	Ala	Asn	Leu	Thr	Cys	Lys	Lys	Pro	Asp
		375					380				
Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	Gln	His	Leu	Pro
385					390					395	
Lys	Arg	Leu	His	Tyr	Ala	Asn	Asn	Arg	Arg	Ile	Glu
			400					405			
Asp	Ile	His	Leu	Leu	Val	Glu	Arg	Arg	Trp	His	Val
410						415					420
Ala	Arg	Lys	Pro	Leu	Asp	Val	Tyr	Lys	Lys	Pro	Ser
				425					430		
Gly	Lys	Cys	Phe	Phe	Gln	Gly	Asp	His	Gly	Phe	Asp
		435					440				
Asn	Lys	Val	Asn	Ser	Met	Gln	Thr	Val	Phe	Val	Gly
445					450					455	
Tyr	Gly	Pro	Thr	Phe	Lys	Tyr	Lys	Thr	Lys	Val	Pro
			460					465			
Pro	Phe	Glu	Asn	Ile	Glu	Leu	Tyr	Asn	Val	Met	Cys
470						475					480
Asp	Leu	Leu	Gly	Leu	Lys	Pro	Ala	Pro	Asn	Asn	Gly
				485					490		
Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Arg	Thr	Asn
		495					500				
Thr	Phe	Arg	Pro	Thr	Met	Pro	Glu	Glu	Val	Thr	Arg
505					510					515	
Pro	Asn	Tyr	Pro	Gly	Ile	Met	Tyr	Leu	Gln	Ser	Asp
			520					525			
Asp	Asp	Leu	Gly	Cys	Thr	Cys	Asp	Asp	Lys	Val	Glu
530						535					540
Pro	Lys	Asn	Lys	Leu	Asp	Glu	Leu	Asn	Lys	Arg	Leu
				545					550		
His	Thr	Lys	Gly	Ser	Thr	Glu	Glu	Arg	His	Leu	Leu
		555					560				
Tyr	Gly	Arg	Pro	Ala	Val	Leu	Tyr	Arg	Thr	Arg	Tyr
565					570					575	
Asp	Ile	Leu	Tyr	His	Thr	Asp	Phe	Glu	Ser	Gly	Tyr
			580					585			
Ser	Glu	Ile	Phe	Leu	Met	Leu	Leu	Trp	Thr	Ser	Tyr
590						595					600
Thr	Val	Ser	Lys	Gln	Ala	Glu	Val	Ser	Ser	Val	Pro
				605						610	

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Asp	His	Leu	Thr	Ser	Cys	Val	Arg	Pro	Asp	Val	Arg
		615					620				
Val	Ser	Pro	Ser	Phe	Ser	Gln	Asn	Cys	Leu	Ala	Tyr
625					630					635	
Lys	Asn	Asp	Lys	Gln	Met	Ser	Tyr	Gly	Phe	Leu	Phe
			640					645			
Pro	Pro	Tyr	Leu	Ser	Ser	Ser	Pro	Glu	Ala	Lys	Tyr
	650					655					660
Asp	Ala	Phe	Leu	Val	Thr	Asn	Met	Val	Pro	Met	Tyr
			665						670		
Pro	Ala	Phe	Lys	Arg	Val	Trp	Asn	Tyr	Phe	Gln	Arg
		675					680				
Val	Leu	Val	Lys	Lys	Tyr	Ala	Ser	Glu	Arg	Asn	Gly
685					690					695	
Val	Asn	Val	Ile	Ser	Gly	Pro	Ile	Phe	Asp	Tyr	Asp
			700					705			
Tyr	Asp	Gly	Leu	His	Asp	Thr	Glu	Asp	Lys	Ile	Lys
	710					715					720
Gln	Tyr	Val	Glu	Gly	Ser	Ser	Ile	Pro	Val	Pro	Thr
				725					730		
His	Tyr	Tyr	Ser	Ile	Ile	Thr	Ser	Cys	Leu	Asp	Phe
		735					740				
Thr	Gln	Pro	Ala	Asp	Lys	Cys	Asp	Gly	Pro	Leu	Ser
745					750					755	
Val	Ser	Ser	Phe	Ile	Leu	Pro	His	Arg	Pro	Asp	Asn
			760					765			
Glu	Glu	Ser	Cys	Asn	Ser	Ser	Glu	Asp	Glu	Ser	Lys
	770					775					780
Trp	Val	Glu	Glu	Leu	Met	Lys	Met	His	Thr	Ala	Arg
				785					790		
Val	Arg	Asp	Ile	Glu	His	Leu	Thr	Ser	Leu	Asp	Phe
		795					800				
Phe	Arg	Lys	Thr	Ser	Arg	Ser	Tyr	Pro	Glu	Ile	Leu
805					810					815	
Thr	Leu	Lys	Thr	Tyr	Leu	His	Thr	Tyr	Glu	Ser	Glu
			820					825			
Ile											

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2946
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Human
 - (B) STRAIN:

- (C) INDIVIDUAL ISOLATE:
- (D) DEVELOPMENTAL STAGE:
- (E) HAPLOTYPE:
- (F) TISSUE TYPE:
- (G) CELL TYPE: Melanoma
- (H) CELL LINE: A2058
- (I) ORGANELLE:

- (ix) FEATURE:
- (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Partial DNA Sequence of A2058 Autotaxin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCTGCCATGA	CTTTGATGAG	CTGTGTTTGA	AGACAGCCCG	40
TGGCTGGGAG	TGTACTAAGG	ACAGATGTGG	AGAAGTCAGA	80
AATGAAGAAA	ATGCCTGTCA	CTGCTCAGAG	GACTGCTTGG	120
CCAGGGGAGA	CTGCTGTACC	AATTACCAAG	TGGTTTGCAA	160
AGGAGAGTCG	CATTGGGTTG	ATGATGACTG	TGAGGAAATA	200
AAGGCCGCAG	AATGCCCTGC	AGGGTTTGTG	CGCCCTCCAT	240
TAATCATCTT	CTCCGTGGAT	GGCTTCCGTG	CATCATACAT	280
GAAGAAAGGC	AGCAAAGTCA	TGCCTAATAT	TGAAAAACTA	320
AGGTCTTG TG	GCACACACTC	TCCCTACATG	AGGCCGGTGT	360
ACCCAATAA	AACCTTTCCT	AACTTATACA	CTTTGGCCAC	400
TGGGCTATAT	CCAGAATCAC	ATGGAATTGT	TGGCAATTCA	440
ATGTATGATC	CTGTATTTGA	TGCCACTTTT	CATCTGCGAG	480
GGCGAGAGAA	ATTTAATCAT	AGATGGTGGG	GAGGTCAACC	520
GCTATGGATT	ACAGCCACCA	AGCAAGGGGT	GAAAGCTGGA	560
ACATTCTTTT	GGTCTGTTGT	CATCCCTCAC	GAGCGGAGAA	600
TATTAACCAT	ATTGCGGTGG	CTCACCTGTC	CAGATCATGA	640
GAGGCCTTCG	GTCTATGCCT	TCTATTCTGA	GCAACCTGAT	680
TTCTCTGGAC	ACAAATATGG	CCCTTTCGGC	CCTGAGGAGA	720
GTAGTTATGG	CTCACCTTTT	ACTCCGGCTA	AGAGACCTAA	760
GAGGAAAGTT	GCCCCTAAGA	GGAGACAGGA	AAGACCAGTT	800
GCTCCTCCAA	AGAAAAGAAG	AAGAAAAATA	CATAGGATGG	840
ATCATTATGC	TGCGGAAACT	CGTCAGGACA	AAATGACAAA	880
TCCTCTGAGG	GAAATCGACA	AAATTGTGGG	GCAATTAATG	920
GATGGACTGA	AACAACTAAA	ACTGCGTCGG	TGTGTCAACG	960
TCATCTTTGT	CGGAGACCAT	GGAATGGAAG	ATGTCACATG	1000
TGATAGAACT	GAGTTCTTGA	GTAATTACCT	AACTAATGTG	1040
GATGATATTA	CTTTAGTGCC	TGGAACCTCTA	GGAAGAATTC	1080
GATCCAAATT	TAGCAACAAT	GCTAAATATG	ACCCCAAAGC	1120
CATTATTGCC	AATCTCACGT	GTAAAAAACC	AGATCAGCAC	1160
TTTAAGCCTT	ACTTGAAACA	GCACCTTCCC	AAACGTTTGC	1200
ACTATGCCAA	CAACAGAAGA	ATTGAGGATA	TCCATTTATT	1240
GGTGGAACGC	AGATGGCATG	TTGCAAGGAA	ACCTTTGGAT	1280
GTTTATAAGA	AACCATCAGG	AAAATGCTTT	TTCCAGGGAG	1320
ACCACGGATT	TGATAACAAG	GTCAACAGCA	TGCAGACTGT	1360
TTTTGTAGGT	TATGGCCCAA	CATTTAAGTA	CAAGACTAAA	1400
GTGCCTCCAT	TTGAAAACAT	TGAACTTTAC	AATGTTATGT	1440
GTGATCTCCT	GGGATTGAAG	CCAGCTCCTA	ATAATGGGAC	1480

CCATGGAAGT	TTGAATCATC	TCCTGCGCAC	TAATACCTTC	1520
AGGCCAACCA	TGCCAGAGGA	AGTTACCAGA	CCCAATTATC	1560
CAGGGATTAT	GTACCTTCAG	TCTGATTTTG	ACCTGGGCTG	1600
CACTTGTGAT	GATAAGGTAG	AGCCAAAGAA	CAAGTTGGAT	1640
GAACTCAACA	AACGGCTTCA	TACAAAAGGG	TCTACAGAAG	1680
AGAGACACCT	CCTCTATGGG	CGACCTGCAG	TGCTTTATCG	1720
GACTAGATAT	GATATCTTAT	ATCACACTGA	CTTTGAAAGT	1760
GGTTATAGTG	AAATATTCCT	AATGCTACTC	TGGACATCAT	1800
ATACTGTTTC	CAAACAGGCT	GAGGTTTCCA	GCGTTCCTGA	1840
CCATCTGACC	AGTTGCGTCC	GGCCTGATGT	CCGTGTTTCT	1880
CCGAGTTTCA	GTCAGAACTG	TTTGGCCTAC	AAAAATGATA	1920
AGCAGATGTC	CTACGGATTG	CTCTTTCCTC	CTTATCTGAG	1960
CTCTTCACCA	GAGGCTAAAT	ATGATGCATT	CCTTGTAACC	2000
AATATGGTTC	CAATGTATCC	TGCTTTCAAA	CGGGTCTGGA	2040
ATTATTTCCA	AAGGGTATTG	GTGAAGAAAT	ATGCTTCGGA	2080
AAGAAATGGA	GTTAACGTGA	TAAGTGGACC	AATCTTCGAC	2120
TATGACTATG	ATGGCTTACA	TGACACAGAA	GACAAAATAA	2160
AACAGTACGT	GGAAGGCAGT	TCCATTCCCTG	TTCCAACTCA	2200
CTACTACAGC	ATCATCACCA	GCTGTCTGGA	TTTCACTCAG	2240
CCTGCCGACA	AGTGTGACGG	CCCTCTCTCT	GTGTCCTCCT	2280
TCATCCTGCC	TCACCGGCCT	GACAAAGAGG	AGAGCTGCAA	2320
TAGCTCAGAG	GACGAATCAA	AATGGGTAGA	AGAACTCATG	2360
AAGATGCACA	CAGCTAGGGT	GCGTGACATT	GAACATCTCA	2400
CCAGCCTGGA	CTTCTTCCGA	AAGACCAGCC	GCAGCTACCC	2440
AGAAATCCTG	ACACTCAAGA	CATACCTGCA	TACATATGAG	2480
AGCGAGATTT	AACTTTCTGA	GCATCTGCAG	TACAGTCTTA	2520
TCAACTGGTT	GTATATTTTT	ATATTGTTTT	TGTATTTATT	2560
AATTTGAAAC	CAGGACATTA	AAAATGTTAG	TATTTTAATC	2600
CTGTACCAAA	TCTGACATAT	TATGCCTGAA	TGACTCCACT	2640
GTTTTTCTCT	AATGCTTGAT	TTAGGTAGCC	TTGTGTTCTG	2680
AGTAGAGCTT	GTAATAAATA	CTGCAGCTTG	AGAAAAAGTG	2720
GAAGCTTCTA	AATGGTGCTG	CAGATTTGAT	ATTTGCATTG	2760
AGGAAATATT	AATTTTCCAA	TGCACAGTTG	CCACATTTAG	2800
TCCTGTACTG	TATGGAAACA	CTGATTTTGT	AAAGTTGCCT	2840
TTATTTGCTG	TTAACTGTTA	ACTATGACAG	ATATATTTAA	2880
GCCTTATAAA	CCAATCTTAA	ACATAATAAA	TCACACATTC	2920
AGTTTTAAAA	AAAAAAAAAA	AAAAAA		2946

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 788
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: No
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Human
 - (B) STRAIN:
 - (C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:
 (E) HAPLOTYPE:
 (F) TISSUE TYPE:
 (G) CELL TYPE: teratocarcinoma
 (H) CELL LINE: N-tera 2D1
 (I) ORGANELLE:

(ix) FEATURE:
 (A) NAME/KEY:
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION: N-tera 2D1 putative
 ATX protein sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Cys	Asp	Asn	Leu	Cys	Lys	Ser	Tyr	Thr	Ser	Cys	Cys
1				5					10		
His	Asp	Phe	Asp	Glu	Leu	Cys	Leu	Lys	Thr	Ala	Arg
		15					20				
Ala	Trp	Glu	Cys	Thr	Lys	Asp	Arg	Cys	Gly	Glu	Val
25					30					35	
Arg	Asn	Glu	Glu	Asn	Ala	Cys	His	Cys	Ser	Glu	Asp
			40					45			
Cys	Leu	Ala	Arg	Gly	Asp	Cys	Cys	Thr	Asn	Tyr	Gln
	50					55					60
Val	Val	Cys	Lys	Gly	Glu	Ser	His	Trp	Val	Asp	Asp
				65					70		
Asp	Cys	Glu	Glu	Ile	Lys	Ala	Ala	Glu	Cys	Leu	Gln
		75					80				
Val	Asp	Ser	Pro	Ser	Ile	Asn	His	Leu	Leu	Arg	Gly
	85				90					95	
Trp	Leu	Pro	Met	Thr	Ser	Tyr	Met	Lys	Lys	Gly	Ser
			100					105			
Lys	Val	Met	Pro	Asn	Ile	Glu	Lys	Leu	Arg	Ser	Cys
	110					115					120
Gly	Thr	His	Ser	Pro	Tyr	Met	Arg	Pro	Val	Tyr	Pro
				125					130		
Thr	Lys	Thr	Phe	Pro	Asn	Leu	Tyr	Thr	Leu	Ala	Thr
		135					140				
Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Val	Gly	Asn
145					150					155	
Ser	Met	Tyr	Asp	Pro	Val	Phe	Asp	Ala	Thr	Phe	His
			160					165			
Leu	Arg	Gly	Arg	Glu	Lys	Phe	Asn	His	Arg	Trp	Trp
	170					175					180
Ala	Gly	Gln	Pro	Leu	Trp	Ile	Thr	Ala	Thr	Lys	Gln
				185					190		
Arg	Gly	Glu	Ser	Trp	Asn	Ile	Leu	Leu	Val	Cys	Cys
		195					200				
His	Pro	Ser	Arg	Ala	Glu	Ile	Leu	Thr	Ile	Leu	Gln
205					210					215	
Trp	Leu	Thr	Leu	Pro	Asp	His	Glu	Arg	Pro	Ser	Val
			220						225		

Tyr	Ala	Phe	Tyr	Ser	Glu	Gln	Pro	Asp	Phe	Ser	Gly
	230					235					240
His	Lys	His	Met	Pro	Phe	Gly	Pro	Glu	Met	Pro	Asn
				245					250		
Pro	Leu	Arg	Glu	Met	His	Lys	Ile	Val	Gly	Gln	Leu
		255					260				
Met	Asp	Gly	Leu	Lys	Gln	Leu	Lys	Leu	His	Arg	Cys
265					270					275	
Val	Asn	Val	Ile	Phe	Val	Glu	Thr	Met	Asp	Gly	Arg
			280					285			
Cys	His	Met	Tyr	Arg	Thr	Glu	Phe	Leu	Ser	Asn	Tyr
	290					295					300
Leu	Thr	Asn	Val	Asp	Asp	Ile	Thr	Leu	Val	Pro	Gly
				305					310		
Thr	Leu	Gly	Arg	Ile	Arg	Ser	Lys	Phe	Ser	Asn	Asn
		315					320				
Ala	Lys	Tyr	Asp	Pro	Lys	Ala	Ile	Ile	Ala	Asn	Leu
325					330					335	
Thr	Cys	Lys	Lys	Pro	Asp	Gln	His	Phe	Lys	Pro	Tyr
			340					345			
Leu	Lys	Gln	His	Leu	Pro	Lys	Arg	Leu	His	Tyr	Ala
	350					355					360
Asn	Asn	Arg	Arg	Ile	Glu	Asp	Ile	His	Leu	Leu	Val
				365					370		
Glu	Arg	Arg	Trp	His	Val	Ala	Arg	Lys	Pro	Leu	Asp
		375					380				
Val	Tyr	Lys	Lys	Pro	Ser	Gly	Asn	Ala	Phe	Ser	Arg
385					390					395	
Glu	Thr	Thr	Ala	Phe	Asp	Asn	Lys	Val	Asn	Ser	Met
			400					405			
Gln	Thr	Val	Phe	Val	Gly	Tyr	Gly	Pro	Thr	Phe	Lys
	410				415						420
Tyr	Lys	Thr	Lys	Val	Pro	Pro	Phe	Glu	Asn	Ile	Glu
			425						430		
Leu	Tyr	Asn	Val	Met	Cys	Asp	Leu	Leu	Gly	Leu	Lys
		435				440					
Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Phe	Ser	Leu	Asn
445					450					455	
His	Leu	Leu	Arg	Thr	Asn	Thr	Phe	Arg	Pro	Thr	Met
			460					465			
Pro	Glu	Glu	Val	Thr	Arg	Pro	Asn	Tyr	Pro	Gly	Ile
	470				475						480
Met	Tyr	Leu	Gln	Ser	Asp	Phe	Asp	Leu	Gly	Cys	Thr
			485						490		
Cys	Asp	Asp	Lys	Val	Glu	Pro	Lys	Asn	Lys	Leu	Asp
		495					500				
Glu	Leu	Asn	Lys	Arg	Leu	His	Thr	Lys	Gly	Ser	Thr
505					510					515	
Glu	Glu	Arg	His	Leu	Leu	Tyr	Gly	Asp	Arg	Pro	Ala
			520					525			
Val	Leu	Tyr	Arg	Thr	Arg	Tyr	Asp	Ile	Leu	Tyr	His
	530					535					540
Thr	Asp	Phe	Glu	Ser	Gly	Tyr	Ser	Glu	Ile	Phe	Leu
				545					550		

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Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Ser	Lys	Gln
		555					560				
Ala	Glu	Val	Ser	Ser	Val	Pro	Asp	His	Leu	Thr	Ser
565					570					575	
Cys	Val	Arg	Pro	Asp	Val	Arg	Val	Ser	Pro	Ser	Phe
			580					585			
Ser	Gln	Asn	Cys	Leu	Ala	Tyr	Lys	Asn	Asp	Lys	Gln
	590					595					600
Met	Ser	Tyr	Gly	Gly	Leu	Gly	Pro	Pro	Tyr	Leu	Ser
			605						610		
Ser	Ser	Pro	Glu	Ala	Lys	Tyr	Asp	Ala	Phe	Leu	Val
		615					620				
Thr	Asn	Met	Val	Pro	Met	Tyr	Pro	Ala	Phe	Lys	Arg
625					630					635	
Val	Trp	Asn	Tyr	Phe	Gln	Arg	Val	Leu	Val	Lys	Lys
			640					645			
Tyr	Ala	Ser	Glu	Arg	Asn	Gly	Val	Asn	Val	Ile	Ser
	650					655					660
Gly	Pro	Ile	Phe	Asp	Tyr	Asp	Tyr	Asp	Gly	Leu	His
			665						670		
Asp	Thr	Glu	Asp	Lys	Ile	Lys	Gln	Tyr	Val	Glu	Gly
		675					680				
Ser	Ser	Ile	Pro	Val	Pro	Thr	His	Tyr	Tyr	Ser	Ile
685					690					695	
Ile	Thr	Ser	Cys	Leu	Asp	Phe	Thr	Gln	Pro	Ala	Asp
			700					705			
Lys	Cys	Asp	Gly	Pro	Leu	Ser	Val	Ser	Ser	Phe	Ile
	710					715					720
Leu	Pro	His	Arg	Pro	Asp	Asn	Glu	Glu	Ser	Cys	Asn
			725						730		
Ser	Ser	Glu	Asp	Glu	Ser	Lys	Trp	Val	Glu	Glu	Leu
		735				740					
Met	Lys	Met	His	Thr	Ala	Arg	Val	Arg	Asp	Ile	Glu
745					750					755	
His	Leu	Thr	Ser	Leu	Asp	Phe	Phe	Arg	Lys	Thr	Ser
			760					765			
Arg	Ser	Tyr	Pro	Glu	Ile	Leu	Thr	Leu	Lys	Thr	Tyr
	770					775					780
Leu	His	Thr	Tyr	Glu	Ser	Glu	Ile				
				785							

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2712
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: teratocarcinoma
(H) CELL LINE: N-tera 2D1
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: N-tera 2D1 ATX DNA
sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TGTGACAACT	TGTGTAAGAG	CTATACCACT	TGCTGCCATG	40
ACTTTGATGA	GCTGTGTTTG	AAGACAGCCC	GTGCGTGCGA	80
GTGTACTAAG	GACAGATGTG	GGAAGTCAG	AAATGAAGAA	120
AATGCCTGTC	ACTGCTCAGA	GGACTGCTTG	GCCAGGGGAG	160
ACTGCTGTAA	CAATTACCAA	GTGGTTTGCA	AAGGAGAGTC	200
GCATTGGGTT	GATGATGACT	GTGAGGAAAT	AAAGGCCGCA	240
GAATGCCTGC	AGGTTTGTTT	GCCCTCCATT	AATCATCTTC	280
TCCGTGGATG	GCTTCCGATG	ACATCATACA	TGAAGAAAGG	320
CAGCAAAGTC	ATGCCTAATA	TTGAAAAACT	AAGGTCTTGT	360
GGCACACACT	CTCCCTACAT	GAGGCCGGTG	TACCCAACTA	400
AAACCTTTCC	TAACTTATAC	ACTTTGGCCA	CTGGGCTATA	440
TCCAGAATCA	CATGGAATTG	TTGGCAATTC	AATGTATGAT	480
CCTGTATTTG	ATGCCACTTT	TCATCTGCGA	GGGCGAGAGA	520
AATTTAATCA	TAGATGGTGG	GGAGGTCAAC	CGCTATGGAT	560
TACAGCCACC	AAGCAAAGGG	GTGAAAGCTG	GAACATTCTT	600
TTGGTCTGTT	GTCATCCCTC	ACGAGCGGAG	ATATTAACCA	640
TATTGCAGTG	GCTCACCTTG	CCAGATCATG	AGAGGCCTTC	680
GGTCTATGCC	TTCTATTCTG	AGCAACCTGA	TTTCTCTGGA	720
CACAAACATA	TGCCTTTCGG	CCCTGAGATG	ACAAATCCTC	760
TGAGGGAAAT	GCACAAAATT	GTGGGGCAAT	TAATGGATGG	800
ACTGAAACAA	CTAAAACCTG	ATCGGTGTGT	CAACGTCATC	840
TTTGTCGAGA	CCATGGATGG	AAGATGTCAC	ATGTATAGAA	880
CTGAGTTCTT	GAGTAATTAC	CTAACTAATG	TGGATGATAT	920
TACTTTAGTG	CCTGGAACCT	TAGGAAGAAT	TCGATCCAAA	960
TTTAGCAACA	ATGCTAAATA	TCACCCCAAA	GCCATTATTG	1000
CCAATCTCAC	GTGTAAAAAA	CCAGATCAGC	ACTTTAAGCC	1040
TTACTTGAAA	CAGCACCTTC	CCAAACGTTT	GCACTATGCC	1080
AACAACAGAA	GAATTGAGGA	TATCCATTTA	TTGGTGGAAC	1120
GCAGATGGCA	TGTTGCAAGG	AAACCTTTGG	ATGTTTATAA	1160
GAAACCATCA	GGAAATGCTT	TTTCCAGGGA	GACCACGGCA	1200
TTTGATAACA	AGGTCAACAG	CATGCAGACT	GTTTTTGTAG	1240
GTTATGGCCC	AACATTTAAG	TACAAGACTA	AAGTDCCTCC	1280
ATTTGAAAAC	ATTGAACTTT	AAAATGTTAT	GTGTGATCTC	1320

CTGGGATTGA	AGCCAGCTCC	TAATAATGGG	ACCCATGGAA	1360
GTTTGAATCA	TCTCCTGCGC	ACTAATACCT	TCAGGCCAAC	1400
CATGCCAGAG	GAAGTTACCA	GACCCTATTA	TCCAGGGATT	1440
ATGTACCTTC	AGTCTGATTT	TGACCTGGGC	TGCACTTGTG	1480
ATGATAAGGT	AGAGCCAAAG	AACAAGTTGG	ATGAACTCAA	1520
CAAACGGCTT	CATACAAAAG	GGTCTACAGA	AGAGAGACAC	1560
CTCCTCTATG	GGGATCGACC	TGCAGTGCTT	TATCGGACTA	1600
GATATGATAT	CTTATATCAC	ACTGACTTTG	AAAGTGGTTA	1640
TAGTGAAATA	TTCTTAATGC	CACTCTGGAC	ATCATATACT	1680
GTTTCCAAAC	AGGCTGAGGT	TTCCAGCGTT	CCTGACCATC	1720
TGACCAGTTG	CGTCCGGCCT	GATGTCCGTG	TTTCTCCGAG	1760
TTTCAGTCAG	AACTGTTTGG	CCTACAAAAA	TGATAAGCAG	1800
ATGTCCTACG	GATTCCTCTT	TCCTCCTTAT	CTGAGCTCTT	1840
CACCAGAGGC	TAAATATGAT	GCATTCCTTG	TAACCAATAT	1880
GGTTCCAATG	TATCCTGCTT	TCAAACGGGT	CTGGAATTAT	1920
TTCCAAAGGG	TATTGGTGAA	GAAATATGCT	TCGGAAAGAA	1960
ATGGAGTTAA	CGTGATAAGT	GGACCAATCT	TCGACTATGA	2000
CTATGATGGC	TTACATGACA	CAGAAGACAA	AATAAAACAG	2040
TACGTGGAAG	GCAGTTCCAT	TCCTGTTCCA	ACTCACTACT	2080
ACAGCATCAT	CACCAGCTGT	CTGGATTTC	CTCAGCCTGC	2120
CGACAAGTGT	GACGGCCCTC	TCTCTGTGTC	CTCCTTCATC	2160
CTGCCTCACC	GGCCTGACAA	CGAGGAGAGC	TGCAATAGCT	2200
CAGAGGACGA	ATCAAAATGG	GTAGAAGAAC	TCATGAAGAT	2240
GCACACAGCT	AGGGTGCGTG	ACATTGAACA	TCTCACCAGC	2280
CTGGACTTCT	TCCGAAAGAC	CAGCCGCAGC	TACCCAGAAA	2320
TCCTGACACT	CAAGACATAC	CTGCATACAT	ATGAGAGCGA	2360
GATTTAACTT	TCTGAGCATC	TGCAGTACAG	TCTTATCAAC	2400
TGGTTGTATA	TTTTTATATT	GTTTTTGTAT	TTATTAATTT	2440
GAAACCAGGA	CATTAAAAAT	GTTAGTATTT	TAATCCTGTA	2480
CCAAATCTGA	CATATTATGC	CTGAATGACT	CCACTGTTTT	2520
TCTCTAATGC	TTGATTTAGG	TAGCCTTGTG	TTCTGAGTAG	2560
AGCTTGTAAT	AAATACTGCA	GCTTGAGTTT	TTAGTGGAAG	2600
CTTCTAAATG	GTGCTGCAGA	TTTGATATTT	GCATTGAGGA	2640
AATATTAATT	TTCCAATGCA	CAGTTGCCAC	ATTTAGTCCT	2680
GTACTGTATG	GAAACACTGA	TTTTGTAAAG	TT	2712

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 979
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: No
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Human
 - (B) STRAIN:
 - (C) INDIVIDUAL ISOLATE:
 - (D) DEVELOPMENTAL STAGE:
 - (E) HAPLOTYPE:

(F) TISSUE TYPE: Liver
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: putative autotaxin
protein sequence from human liver

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met	Ala	Arg	Arg	Ser	Ser	Phe	Gln	Ser	Cys	Gln	Asp
1				5					10		
Ile	Ser	Leu	Phe	Thr	Phe	Ala	Val	Gly	Val	Asn	Ile
		15					20				
Cys	Leu	Gly	Phe	Thr	Ala	His	Arg	Ile	Lys	Arg	Ala
25					30					35	
Glu	Gly	Trp	Glu	Glu	Gly	Pro	Pro	Thr	Val	Leu	Ser
			40					45			
Asp	Ser	Pro	Trp	Thr	Asn	Ile	Ser	Gly	Ser	Cys	Lys
	50					55					60
Gly	Arg	Cys	Phe	Glu	Leu	Gln	Glu	Ala	Gly	Pro	Pro
			65						70		
Asp	Cys	Arg	Cys	Asp	Asn	Leu	Cys	Lys	Ser	Tyr	Thr
		75					80				
Ser	Cys	Cys	His	Asp	Phe	Asp	Glu	Leu	Cys	Leu	Lys
85					90					95	
Thr	Ala	Arg	Ala	Trp	Glu	Cys	Thr	Lys	Asp	Arg	Cys
			100					105			
Gly	Glu	Val	Arg	Asn	Glu	Glu	Asn	Ala	Cys	His	Cys
	110					115					120
Ser	Glu	Asp	Cys	Leu	Ala	Arg	Gly	Asp	Cys	Cys	Thr
			125						130		
Asn	Tyr	Gln	Val	Val	Cys	Lys	Gly	Glu	Ser	His	Trp
	135						140				
Val	Asp	Asp	Asp	Cys	Glu	Glu	Ile	Lys	Ala	Ala	Glu
145					150					155	
Cys	Leu	Gln	Val	Cys	Ser	Pro	Ser	Ile	Asn	His	Leu
			160					165			
Leu	Arg	Gly	Trp	Leu	Pro	Met	Thr	Ser	Tyr	Met	Lys
	170					175					180
Lys	Gly	Ser	Lys	Val	Met	Pro	Asn	Ile	Glu	Lys	Leu
			185						190		
Arg	Ser	Cys	Gly	Thr	His	Ser	Pro	Tyr	Met	Arg	Pro
		195					200				
Val	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	Leu	Tyr	Thr
205					210					215	
Leu	Ala	Thr	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile
			220					225			
Val	Gly	Asn	Ser	Met	Tyr	Asp	Pro	Val	Phe	Asp	Ala
	230					235					240

Thr	Phe	His	Leu	Arg	Gly	Arg	Glu	Lys	Phe	Asn	His	
				245					250			
Arg	Trp	Trp	Gly	Gly	Gln	Pro	Leu	Trp	Ile	Thr	Ala	
		255					260					
Thr	Lys	Gln	Arg	Gly	Glu	Ser	Trp	Asn	Ile	Leu	Leu	
265					270					275		
Val	Cys	Cys	His	Pro	Ser	Arg	Ala	Glu	Ile	Leu	Thr	
			280					285				
Ile	Leu	Gln	Trp	Leu	Thr	Leu	Pro	Asp	His	Glu	Arg	
	290					295					300	
Pro	Ser	Val	Tyr	Ala	Phe	Tyr	Ser	Glu	Gln	Pro	Asp	
				305					310			
Phe	Ser	Gly	His	Lys	His	Met	Pro	Phe	Gly	Pro	Glu	
		315					320					
Met	Thr	Asn	Pro	Leu	Arg	Glu	Met	His	Lys	Ile	Val	
325					330					335		
Gly	Gln	Leu	Met	Asp	Gly	Leu	Lys	Gln	Leu	Lys	Leu	
			340					345				
His	Arg	Cys	Val	Asn	Val	Ile	Phe	Val	Glu	Thr	Met	
	350					355					360	
Asp	Gly	Arg	Cys	His	Met	Tyr	Arg	Thr	Glu	Phe	Leu	
				365					370			
Ser	Asn	Tyr	Leu	Thr	Asn	Val	Asp	Asp	Ile	Thr	Leu	
		375					380					
Val	Pro	Gly	Thr	Leu	Gly	Arg	Ile	Arg	Ser	Lys	Phe	
385					390					395		
Ser	Asn	Asn	Ala	Lys	Tyr	Asp	Pro	Lys	Ala	Ile	Ile	
			400					405				
Ala	Asn	Leu	Thr	Cys	Lys	Lys	Pro	Asp	Gln	His	Phe	
	410					415					420	
Lys	Pro	Tyr	Leu	Lys	Gln	His	Leu	Pro	Lys	Arg	Leu	
				425					430			
His	Tyr	Ala	Asn	Asn	Arg	Arg	Ile	Glu	Asp	Ile	His	
		435					440					
Leu	Leu	Val	Glu	Arg	Arg	Trp	His	Val	Ala	Arg	Lys	
445					450					455		
Pro	Leu	Asp	Val	Tyr	Lys	Lys	Pro	Ser	Gly	Asn	Ala	
			460					465				
Phe	Ser	Arg	Glu	Thr	Thr	Ala	Phe	Asp	Asn	Lys	Val	
	470					475					480	
Asn	Ser	Met	Gln	Thr	Val	Phe	Val	Gly	Tyr	Gly	Pro	
				485					490			
Thr	Phe	Lys	Tyr	Lys	Thr	Lys	Val	Pro	Pro	Phe	Glu	
		495					500					
Asn	Ile	Glu	Leu	Tyr	Asn	Val	Met	Cys	Asp	Leu	Leu	
505					510					515		
Gly	Leu	Lys	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	
			520					525				
Ser	Leu	Asn	His	Leu	Leu	Arg	Thr	Asn	Thr	Phe	Arg	
	530					535					540	
Pro	Thr	Met	Pro	Glu	Glu	Val	Thr	Arg	Pro	Asn	Tyr	
				545					550			
Pro	Gly	Ile	Met	Tyr	Leu	Gln	Ser	Asp	Phe	Asp	Leu	
		555					560					

004634.03400

Gly 565	Cys	Thr	Cys	Asp	Asp 570	Lys	Val	Glu	Pro	Lys 575	Asn
Lys	Leu	Asp	Glu 580	Leu	Asn	Lys	Arg	Leu 585	His	Thr	Lys
Gly 590	Ser	Thr	Glu	Glu	Arg	His 595	Leu	Leu	Tyr	Gly	Asp 600
Arg	Pro	Ala	Val	Leu 605	Tyr	Arg	Thr	Arg	Tyr 610	Asp	Ile
Leu	Tyr	His 615	Thr	Asp	Phe	Glu	Ser 620	Gly	Tyr	Ser	Glu
Ile 625	Phe	Leu	Met	Pro	Leu 630	Trp	Thr	Ser	Tyr	Thr	Val 635
Ser	Lys	Gln	Ala 640	Glu	Val	Ser	Ser	Val 645	Pro	Asp	His
Leu 650	Thr	Ser	Cys	Val	Arg	Pro 655	Asp	Val	Arg	Val	Ser 660
Pro	Ser	Phe	Ser	Gln 665	Asn	Cys	Leu	Ala	Tyr 670	Lys	Asn
Asp	Lys	Gln 675	Met	Ser	Tyr	Gly	Phe 680	Leu	Phe	Pro	Pro
Tyr 685	Leu	Ser	Ser	Ser	Pro 690	Glu	Ala	Lys	Tyr	Asp	Ala 695
Phe	Leu	Val 700	Thr	Asn	Met	Val	Pro	Met 705	Tyr	Pro	Ala
Phe 710	Lys	Arg	Val	Trp	Asn	Tyr 715	Phe	Gln	Arg	Val	Leu 720
Val	Lys	Lys	Tyr 725	Ala	Ser	Glu	Arg	Asn	Gly 730	Val	Asn
Val	Ile	Ser 735	Gly	Pro	Ile	Phe	Asp 740	Tyr	Asp	Tyr	Asp
Gly 745	Leu	His	Asp	Thr	Glu 750	Asp	Lys	Ile	Lys	Gln	Tyr 755
Val	Glu	Gly 760	Ser	Ser	Ile	Pro	Val	Pro 765	Thr	His	Tyr
Tyr 770	Ser	Ile	Ile	Thr	Ser	Cys 775	Leu	Asp	Phe	Thr	Gln 780
Pro	Ala	Asp	Lys 785	Cys	Asp	Gly	Pro	Leu	Ser 790	Val	Ser
Ser	Phe 795	Ile	Leu	Pro	His	Arg	Pro 800	Asp	Asn	Glu	Glu
Ser 805	Cys	Asn	Ser	Ser	Glu 810	Asp	Glu	Ser	Lys	Trp	Val 815
Glu	Glu	Leu	Met 820	Lys	Met	His	Thr	Ala 825	Arg	Val	Arg
Asp 830	Ile	Glu	His	Leu	Thr	Ser 835	Leu	Asp	Phe	Phe	Arg 840
Lys	Thr	Ser	Arg	Ser 845	Tyr	Pro	Glu	Ile	Leu	Thr	Leu 850
Lys	Thr	Tyr 855	Leu	His	Thr	Tyr	Glu 860	Ser	Glu	Ile	Xaa
Leu 865	Ser	Glu	His	Leu	Gln 870	Tyr	Ser	Leu	Ile	Asn	Trp 875
Leu	Tyr	Ile	Phe 880	Ile	Leu	Phe	Leu	Tyr 885	Leu	Leu	Ile

Xaa	Asn	Gln	Asp	Ile	Lys	Asn	Val	Ser	Ile	Leu	Ile
	890					895					900
Leu	Tyr	Gln	Ile	Xaa	His	Ile	Met	Pro	Glu	Xaa	Leu
				905					910		
His	Cys	Phe	Ser	Leu	Met	Leu	Asp	Leu	Gly	Ser	Leu
		915					920				
Val	Phe	Xaa	Val	Glu	Leu	Val	Ile	Asn	Thr	Ala	Ala
925					930					935	
Xaa	Val	Phe	Ser	Gly	Ser	Phe	Xaa	Met	Val	Leu	Gln
			940					945			
Ile	Xaa	Tyr	Leu	His	Xaa	Gly	Asn	Ile	Asn	Phe	Pro
	950					955					960
Met	His	Ser	Cys	His	Ile	Xaa	Ser	Cys	Thr	Val	Trp
				965					970		
Lys	His	Xaa	Phe	Cys	Lys	Val					
		975									

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8
 - (B) TYPE: amino acids
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
 - (A) DESCRIPTION: peptide
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
 - (A) NAME/KEY: ATX-204
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met His Thr Ala Arg Val Arg Asp
5

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: No

- (ix) FEATURE:
(A) NAME/KEY: ATX-205
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Phe Ser Asn Asn Ala Lys Tyr Asp
5

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
(A) NAME/KEY: ATX-209
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Val Met Pro Asn Ile Glu Lys
5

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
(A) NAME/KEY: ATX-210
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Thr Ala Arg Gly Trp Glu Cys Thr
5

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-212
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Xaa Asp Ser Pro Trp Thr Xaa Ile Ser Gly Ser
5 10

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-214
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Leu Arg Ser Cys Gly Thr His Ser Pro Tyr Met
5 10

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
 - (A) DESCRIPTION: Peptide
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
 - (A) NAME/KEY: ATX-215/34A
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Thr Tyr Leu His Thr Tyr Glu Ser
5

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13
 - (B) TYPE: amino acids
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
 - (A) DESCRIPTION: Peptide
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Ala Ile Ile Ala Asn Leu Thr Cys Lys Lys Pro Asp Gln
5 10

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8
 - (B) TYPE: amino acids
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
 - (A) DESCRIPTION: Peptide
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
 - (A) NAME/KEY: ATX-216
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Ile Val Gly Gln Leu Met Asp Gly
5

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9
 - (B) TYPE: amino acids
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
 - (A) DESCRIPTION: Peptide
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
 - (A) NAME/KEY: ATX-218/44
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Thr Ser Arg Ser Tyr Pro Glu Ile Leu
5

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9

(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-223B/24
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gln Ala Glu Val Ser Ser Val Pro Asp
5

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-224
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Cys Phe Glu Leu Gln Glu Ala Gly Pro Pro Asp Asp Cys
5 10

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:

(A) NAME/KEY: ATX-229

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Ser Tyr Thr Ser Cys Cys His Asp Phe Asp Glu Leu

5 10

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:

(A) NAME/KEY: ATX-224/53

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gln Met Ser Tyr Gly Phe Leu Phe Pro Pro Tyr Leu

1 5 10

Ser Ser Ser Pro

15

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: cDNA

- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE:
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Human
 - (B) STRAIN:
 - (C) INDIVIDUAL ISOLATE:
 - (D) DEVELOPMENTAL STAGE:
 - (E) HAPLOTYPE:
 - (F) TISSUE TYPE: Liver
 - (G) CELL TYPE:
 - (H) CELL LINE:
 - (I) ORGANELLE:
- (ix) FEATURE:
- (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: 5' end of human liver
ATX gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ATGGCAAGGA	GGAGCTCGTT	CCAGTCGTGT	CAAGATATAT	40
CCCTGTTTAC	TTTGCCGTT	GGAGTCAATA	TCTGCTTAGG	80
ATTCAGTGCA	CATCGAATTA	AGAGAGCAGA	AGGATGG	117

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 39
 - (B) TYPE: amino acids
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE:
- (A) DESCRIPTION: Peptide
- (iii) HYPOTHETICAL: No
- (v) FRAGMENT TYPE: N-terminal fragment
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Human
 - (B) STRAIN:
 - (C) INDIVIDUAL ISOLATE:
 - (D) DEVELOPMENTAL STAGE:
 - (E) HAPLOTYPE:
 - (F) TISSUE TYPE: Liver
 - (G) CELL TYPE:
 - (H) CELL LINE:
 - (I) ORGANELLE:

- (ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: N-terminal region
including transmembrane domain of liver
ATX protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Asp
1 5 10
Ile Ser Leu Phe Thr Phe Ala Val Gly Val Asn Ile
15 20
Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala
25 30 35
Glu Gly Trp

- (2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Primer from 5' end of
4C11

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GCTCAGATAA GGAGGAAAGA G

21

- (2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Nested primers from 4C11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GAATCCGTAG GACATCTGCT T

21

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Nested primers from 4C11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TGTAGGCCAA ACAGTTCTGA C

21

(2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Nested sense primer deduced from ATX-101, wherein N is inosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

AAYTCNATGC ARACNGTNTT YGTNG

25

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Nested primer of ATX -101, wherein N is inosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TTYGTNGGNT AYGGNCCNAC NTTYAA

26

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Nested primer deduced from ATX-103, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

AAYTAYCTNA CNAAYGTNGA YGAYAT

26

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Nested primer deduced from ATX-103, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GAYGAYATNA CNCTNGTNCC NGGNAC

26

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

- (ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested primer deduced
from ATX-103, wherein N is inosine

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TGYTTYGARY TNCARGARGC NGGNCCNCC

29

- (2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: No

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCTGTCTTCA AACACAGC

18

- (2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: No

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CTGGTGGCTG TAATCCATAG C

21

- (2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Primer for 5' end of N-tera 2D1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CGTGAAGGCA AAGAGAACAC G

21

(2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3104
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
 - (A) NAME/KEY: N-tera 2D1 ATX cDNA
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

AGTGCACTCC	GTGAAGGCAA	AGAGAACACG	CTGCAAAAGG	40
CTTTCCAATA	ATCCTCGACA	TGGCAAGGAG	GAGCTCGTTC	80
CAGTCGTGTC	AGATAATATC	CCTGTTCAC	TTTGCCGTTG	120
GAGTCAATAT	CTGCTTAGGA	TTCCTGCAC	ATCGAATTAA	160
GAGAGCAGAA	GGATGGGAGG	AAGGTCCTCC	TACAGTGCTA	200
TCAGACTCCC	CCTGGACCAA	CATCTCCGGA	TCTTGCAAGG	240
GCAGGTGCTT	TGAACTTCAA	GAGGCTGGAC	CTCCTGATTG	280
TCGCTGTGAC	AACTTGTGTA	AGAGCTATAC	CAGTTGCTGC	320
CATGACTTTG	ATGAGCTGTG	TTTGAAGACA	GCCCGTGCGT	360
GGGAGTGTAC	TAAGGACAGA	TGTGGAGAAG	TCAGAAATGA	400
AGAAAATGCC	TGTCACTGCT	CAGAGGACTG	CTTGGCCAGG	440
GGAGACTGCT	GTACCAATTA	CCAAGTGGTT	TGCAAAGGAG	480
AGTCGCATTG	GGTTGATGAT	GACTGTGAGG	AAATAAAGGC	520
CGCAGAATGC	CCTGCAGGGT	TTGTTCGCCC	TCCATTAATC	560

ATCTTCTCCG	TGGATGGCTT	CCGTGCATCA	TACATGAAGA	600
AAGGCAGCAA	AGTCATGCCT	AATATTGAAA	AACTAAGGTC	640
TTGTGGCACA	CACTCGCCCC	ACATGAGGCC	GGTGTACCCA	680
ACTAAAACCT	TTCCTAACTT	ATACACTTTG	GCCACTGGGC	720
TATATCCAGA	ATCACATGGA	ATTGTTGGCA	ATTCAATGTA	760
TGATCCTGTA	TTTGATGCCA	CTTTTCATCT	GCGAGGGCGA	800
GAGAAATTTA	ATCATAGATG	GTGGGGAGGT	CAACCGCTAT	840
GGATTACAGC	CACCAAGCAA	AGGGGTGAAA	GCTGGAACAT	880
TCTTTTGGTC	TGTTGTCATC	CCTCACGAGC	GGAGATATTA	920
ACCATATTGC	AGTGGCTCAC	CCTGCCAGAT	CATGAGAGGC	960
TTCGGTCTAT	GCCTTCTATT	CTGAGCAACC	TGATTTCTCT	1000
GGACACAAAT	ATGCCTTTCG	GCCCTGAGAT	GACAAATCCT	1040
CTGAGGGAAA	TCGACAAAAT	TGTGGGGCAA	TTAATGGATG	1080
GACTGAAACA	ACTAAAACCTG	CATCGGTGTG	TCAACGTCAT	1120
CTTTGTCTGA	GACCATGGAA	TGGAAGATGT	CACATGTGAT	1160
AGAACTGAGT	TCTTGAGTAA	TTACCTAACT	AATGTGGATG	1200
ATATTACTTT	AGTGCCTGGA	ACTCTAGGAA	TTCGATCCAA	1240
ATTTAGCAAC	AATGCTAAAT	ATGACCCCAA	AGCCATTATT	1280
GCCAATCTCA	CGTGTAAGAA	ACCAGATCAG	CACTTTAAGC	1320
CTTACTTGAA	ACAGCACCTT	CCCAAACGTT	TGCACTATGC	1360
CAACAACAGA	AGAATTGAGG	ATATCCATTT	ATTGGTGGA	1400
CGCAGATGGC	ATGTTGCAAG	GAAACCTTTG	GATGTTTATA	1440
AGAAACCATC	AGGAAAATGC	TTTTTCCAGG	GAGACCACGG	1480
ATTTGATAAC	AAGGTCAACA	GCATGCAGAC	TGTTTTTTGTA	1520
GGTTATGGCC	CAACATTTAA	GTACAAGACT	AAAGTGCCTC	1560
CATTTGAAAA	CATTGAACTT	TACAATGTTA	TGTGTGATCT	1600
CCTGGGATTG	AAGCCAGCTC	CTAATAATGG	GACCCATGGA	1640
AGTTTGAATC	ATCTCCTGCG	CACTAATACC	TTCAGGCCAA	1680
CCATGCCAGA	GGAAGTTACC	AGACCCAATT	ATCCAGGGAT	1720
TATGTACCTT	CAGTCTGATT	TTGACCTGGG	CTGCACTTGT	1760
GATGATAAGG	TAGAGCCAAA	GAACAAGTTG	GATGAACTCA	1800
ACAAACGGCT	TCATACAAAA	GGGTCTACAG	AAGAGAGACA	1840
CCTCCTCTAT	GGGCGACCTG	CAGTGCTTTA	TCGGACTAGA	1880
TATGATGTCT	TATATCACAC	TGACTTTGAA	AGTGGTTATA	1920
GTGAAATATT	CCTAATGCCA	CTCTGGACAT	CATATACTGT	1960
TTCCAAACAG	GCTGAGGTTT	CCAGCGTTCC	TGACCATCTG	2000
ACCAGTTGCG	TCCGGCCTGA	TGTCCGTGTT	TCTCCGAGTT	2040
TCAGTCAGAA	CTGTTTGGCC	TACAAAAATG	ATAAGCAGAT	2080
GTCCTACGGA	TTCTCTTTTC	CTCCTTATCT	GAGCTCTTCA	2120
CCAGAGGCTA	AATATGATGC	ATTCCTTGTA	ACCAATATGG	2160
TTCCAATGTA	TCCTGCTTTT	AAACGGGTCT	GGAATTATTT	2200
CCAAAGGGTA	TTGGTGAAGA	AATATGCTTC	GGAAAGAAAT	2240
GGAGTTAACG	TGATAAGTGG	ACCAATCTTC	GACTATGACT	2280
ATGATGGCTT	ACATGACACA	GAAGACAAAA	TAAAACAGTA	2320
CGTGGAAGGC	AGTTCCATTC	CTGTTCCAAC	TCACTACTAC	2360
AGCATCATCA	CCAGCTGTCT	GGATTTCACT	CAGCCTGCCG	2400
ACAAGTGTGA	CGGCCCTCTC	TCTGTGTCCT	CCTTCATCCT	2440
CCGTCACCGG	CCTGACAACG	AGGAGAGCTG	CAATAGCTCA	2480
GAGGACGAAT	CAAAATGGGT	AGAAGAACTC	ATGAAGATGC	2520
ACACGGCTAG	GGTGCGTGAC	ATTGAACATC	TCACCAGCCT	2560
GGACTTCTTC	CGAAAGACCA	GCCGCAGCTA	CCCAGAAATC	2600
CTGACACTCA	AGACATACCT	GCATACATAT	GAGAGCGAGA	2640
TTTAACTTTC	TGAGCATCTG	CAGTACAGTC	TTATCAACTG	2680
GTTGTATATT	TTTATATTGT	TTTTGTATTT	ATTAATTGTA	2720

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AACCAGGACA	TTAAAAATGT	TAGTATTTTA	ATCCTGTACC	2760
AAATCTGACA	TATTATGCCT	GAATGACTCC	ACTGTTTTTC	2800
TCTAATGCTT	GATTTAGGTA	GCCTTGTGTT	CTGAGTAGAG	2840
CTTGTAATAA	ATACTGCAGC	TTGAGTTTTT	AGTGGAAGCT	2880
TCTAAATGGT	GCTGCAGATT	TGATATTTGC	ATTGAGGAAA	2920
TATTAATTTT	CCAATGCACA	GTTGCCACAT	TTAGTCCTGT	2960
ACTGTATGGA	AACACTGATT	TTGTAAAGTT	GCCTTTATTT	3000
GCTGTTAACT	GTAACTATG	ACAGATATAT	TTAAGCCTTA	3040
TAAACCAATC	TTAAACATAA	TAAATCACAC	ATTCAGTTTT	3080
TTCTGGTAAA	AAAAAAAAAA	AAAA		3104

(2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 861
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: No
- (ix) FEATURE:
 - (A) NAME/KEY: N-tera 2D1 ATX protein
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Met	Ala	Arg	Arg	Ser	Ser	Phe	Gln	Ser	Cys	Gln	Ile	Ile	Ser	Leu	Phe
1				5					10					15	
Thr	Phe	Ala	Val	Gly	Val	Asn	Ile	Cys	Leu	Gly	Phe	Thr	Ala	His	Arg
			20					25					30		
Ile	Lys	Arg	Ala	Glu	Gly	Trp	Glu	Gly	Pro	Pro	Thr	Val	Leu	Ser	
		35				40					45				
Asp	Ser	Pro	Trp	Thr	Asn	Ile	Ser	Gly	Ser	Cys	Lys	Gly	Arg	Cys	Phe
	50				55					60					
Glu	Leu	Gln	Glu	Ala	Gly	Pro	Pro	Asp	Cys	Arg	Cys	Asp	Asn	Leu	Cys
65					70					75				80	
Lys	Ser	Tyr	Thr	Ser	Cys	Cys	His	Asp	Phe	Asp	Glu	Leu	Cys	Leu	Lys
				85					90					95	
Thr	Ala	Arg	Ala	Trp	Glu	Cys	Thr	Lys	Asp	Arg	Cys	Gly	Glu	Val	Arg
			100					105				110			
Asn	Glu	Glu	Asn	Ala	Cys	His	Cys	Ser	Glu	Asp	Cys	Leu	Ala	Arg	Gly
		115					120					125			
Asp	Cys	Cys	Thr	Asn	Tyr	Gln	Val	Val	Cys	Lys	Gly	Glu	Ser	His	Trp
	130					135					140				
Val	Asp	Asp	Asp	Cys	Glu	Glu	Ile	Lys	Ala	Ala	Glu	Cys	Pro	Ala	Gly
145					150					155					160

Phe	Val	Arg	Pro	Pro	Leu	Ile	Ile	Phe	Ser	Val	Asp	Gly	Phe	Arg	Ala
				165					170					175	
Ser	Tyr	Met	Lys	Lys	Gly	Ser	Lys	Val	Met	Pro	Asn	Ile	Glu	Lys	Leu
			180					185					190		
Arg	Ser	Cys	Gly	Thr	His	Ser	Pro	His	Met	Arg	Pro	Val	Tyr	Pro	Thr
		195					200					205			
Lys	Thr	Phe	Pro	Asn	Leu	Tyr	Thr	Leu	Ala	Thr	Gly	Leu	Tyr	Pro	Glu
	210					215					220				
Ser	His	Gly	Ile	Val	Gly	Asn	Ser	Met	Tyr	Asp	Pro	Val	Phe	Asp	Ala
225					230					235					240
Thr	Phe	His	Leu	Arg	Gly	Arg	Glu	Lys	Phe	Asn	His	Arg	Trp	Trp	Gly
				245					250					255	
Gly	Gln	Pro	Leu	Trp	Ile	Thr	Ala	Thr	Lys	Gln	Arg	Gly	Glu	Ser	Trp
			260					265					270		
Asn	Ile	Leu	Leu	Val	Cys	Cys	His	Pro	Ser	Arg	Ala	Glu	Ile	Leu	Thr
		275					280					285			
Ile	Leu	Gln	Trp	Leu	Thr	Leu	Pro	Asp	His	Glu	Arg	Leu	Arg	Ser	Met
	290					295					300				
Pro	Ser	Ile	Leu	Ser	Asn	Leu	Ile	Ser	Leu	Asp	Thr	Asn	Met	Pro	Phe
305					310					315					320
Gly	Pro	Glu	Met	Thr	Asn	Pro	Leu	Arg	Glu	Ile	Asp	Lys	Ile	Val	Gly
				325					330					335	
Gln	Leu	Met	Asp	Gly	Leu	Lys	Gln	Leu	Lys	Leu	His	Arg	Cys	Val	Asn
			340					345					350		
Val	Ile	Phe	Val	Gly	Asp	His	Gly	Met	Glu	Asp	Val	Thr	Cys	Asp	Arg
		355					360					365			
Thr	Glu	Phe	Leu	Ser	Asn	Tyr	Leu	Thr	Asn	Val	Asp	Asp	Ile	Thr	Leu
	370					375					380				
Val	Pro	Gly	Thr	Leu	Gly	Ile	Arg	Ser	Lys	Phe	Ser	Asn	Asn	Ala	Lys
385					390					395					400
Tyr	Asp	Pro	Lys	Ala	Ile	Ile	Ala	Asn	Leu	Thr	Cys	Lys	Lys	Pro	Asp
				405					410					415	
Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	Gln	His	Leu	Pro	Lys	Arg	Leu	His
			420					425					430		
Tyr	Ala	Asn	Asn	Arg	Arg	Ile	Glu	Asp	Ile	His	Leu	Leu	Val	Glu	Arg
		435					440					445			
Arg	Trp	His	Val	Ala	Arg	Lys	Pro	Leu	Asp	Val	Tyr	Lys	Lys	Pro	Ser
	450					455					460				
Gly	Lys	Cys	Phe	Phe	Gln	Gly	Asp	His	Gly	Phe	Asp	Asn	Lys	Val	Asn
465					470					475					480
Ser	Met	Gln	Thr	Val	Phe	Val	Gly	Tyr	Gly	Pro	Thr	Phe	Lys	Tyr	Lys
				485					490					495	
Thr	Lys	Val	Pro	Pro	Phe	Glu	Asn	Ile	Glu	Leu	Tyr	Asn	Val	Met	Cys
			500					505					510		
Asp	Leu	Leu	Gly	Leu	Lys	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly	Ser
		515					520					525			
Leu	Asn	His	Leu	Leu	Arg	Thr	Asn	Thr	Phe	Arg	Pro	Thr	Met	Pro	Glu
	530					535					540				
Glu	Val	Thr	Arg	Pro	Asn	Tyr	Pro	Gly	Ile	Met	Tyr	Leu	Gln	Ser	Asp
445					450					555					560
Phe	Asp	Leu	Gly	Cys	Thr	Cys	Asp	Asp	Lys	Val	Glu	Pro	Lys	Asn	Lys
				565					570					575	
Leu	Asp	Glu	Leu	Asn	Lys	Arg	Leu	His	Thr	Lys	Gly	Ser	Thr	Glu	Glu
			580					585						590	

Arg	His	Leu	Leu	Tyr	Gly	Arg	Pro	Ala	Val	Leu	Tyr	Arg	Thr	Arg	Tyr
		595					600					605			
Asp	Val	Leu	Tyr	His	Thr	Asp	Phe	Glu	Ser	Gly	Tyr	Ser	Glu	Ile	Phe
	610					615					620				
Leu	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val	Ser	Lys	Gln	Ala	Glu	Val
625					630					635					640
Ser	Ser	Val	Pro	Asp	His	Leu	Thr	Ser	Cys	Val	Arg	Pro	Asp	Val	Arg
				645					650					655	
Val	Ser	Pro	Ser	Phe	Ser	Gln	Asn	Cys	Leu	Ala	Tyr	Lys	Asn	Asp	Lys
			660					665					670		
Gln	Met	Ser	Tyr	Gly	Phe	Leu	Phe	Pro	Pro	Tyr	Leu	Ser	Ser	Ser	Pro
		675					680					685			
Glu	Ala	Lys	Tyr	Asp	Ala	Phe	Leu	Val	Thr	Asn	Met	Val	Pro	Met	Tyr
	690					695					700				
Pro	Ala	Phe	Lys	Arg	Val	Trp	Asn	Tyr	Phe	Gln	Arg	Val	Leu	Val	Lys
705					710					715					720
Lys	Tyr	Ala	Ser	Glu	Arg	Asn	Gly	Val	Asn	Val	Ile	Ser	Gly	Pro	Ile
				725					730					735	
Phe	Asp	Tyr	Asp	Tyr	Asp	Gly	Leu	His	Asp	Thr	Glu	Asp	Lys	Ile	Lys
			740					745					750		
Gln	Tyr	Val	Glu	Gly	Ser	Ser	Ile	Pro	Val	Pro	Thr	His	Tyr	Tyr	Ser
		755					760					765			
Ile	Ile	Thr	Ser	Cys	Leu	Asp	Phe	Thr	Gln	Pro	Ala	Asp	Lys	Cys	Asp
	770					775					780				
Gly	Pro	Leu	Ser	Val	Ser	Ser	Phe	Ile	Leu	Arg	His	Arg	Pro	Asp	Asn
785					790					795					800
Glu	Glu	Ser	Cys	Asn	Ser	Ser	Glu	Asp	Glu	Ser	Lys	Trp	Val	Glu	Glu
			805						810					815	
Leu	Met	Lys	Met	His	Thr	Ala	Arg	Val	Arg	Asp	Ile	Glu	His	Leu	Thr
			820					825					830		
Ser	Leu	Asp	Phe	Phe	Arg	Lys	Thr	Ser	Arg	Ser	Tyr	Pro	Glu	Ile	Leu
		835					840					845			
Thr	Leu	Lys	Thr	Tyr	Leu	His	Thr	Tyr	Glu	Ser	Glu	Ile			
	850					855					860				

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3251
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(ix) FEATURE:

- (A) NAME/KEY: A2058 ATX cDNA
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CGTGAAGGCA	AAGAGAACAC	GCTGCAAAAAG	GCTTCCAAGA	40
ATCCTCGACA	TGGCAAGGAG	GAGCTCGTTC	CAGTCGTGTC	80
AGATAATATC	CCTGTTCACT	TTTGCCGTTG	GAGTCAGTAT	120
CTGCTTAGGA	TTCAC TGAC	ATCGAATTAA	GAGAGCAGAA	160
GGATGGGAGG	AAGGTCCTCC	TACAGTGCTA	TCAGACTCCC	200
CCTGGACCAA	CATCTCCGGA	TCTTGCAAGG	GCAGGTGCTT	240
TGAACTTCAA	GAGGCTGGAC	CTCCTGATTG	TCGCTGTGAC	280
AACTTG TGTA	AGAGCTATAC	CAGTTGCTGC	CATGACTTTG	320
ATGAGCTGTG	TTTGAAGACA	GCCCGTGGCT	GGGAGTGTAC	360
TAAGGACAGA	TGTGGAGAAG	TCAGAAATGA	AGAAAATGCC	400
TGTCACTGCT	CAGAGGACTG	CTTGGCCAGG	GGAGACTGCT	440
GTACCAATTA	CCAAGTGGTT	TGCAAAGGAG	AGTCGCATTG	480
GGTTGATGAT	GACTGTGAGG	AAATAAAGGC	CGCAGAATGC	520
CCTGCAGGGT	TTGTTGCGCC	TCCATTAATC	ATCTTCTCCG	560
TGGATGGCTT	CCGTGCATCA	TACATGAAGA	AAGGCAGCAA	600
AGTCATGCCT	AATATTGAAA	AÀCTAAGGTC	TTGTGGCACA	640
CACTCTCCCT	ACATGAGGCC	GGTGTACCCA	ACTAAAACCT	680
TTCCTAACTT	ATACACTTTG	GCCACTGGGC	TATATCCAGA	720
ATCACATGGA	ATTGTTGGCA	ATTCAATGTA	TGATCCTGTA	760
TTTGATGCCA	CTTTTCATCT	GCGAGGGCGA	GAGAAATTTA	800
ATCATAGATG	GTGGGGAGGT	CAACCGCTAT	GGATTACAGC	840
CACCAAGCAA	GGGGTGAAAG	CTGGAACATT	CTTTTG GTCT	880
GTTGTCATCC	CTCACGAGCG	GAGAATATTA	ACCATATTGC	920
GGTGGCTCAC	CCTGCCAGAT	CATGAGAGGC	CTTCGGTCTA	960
TGCCTTCTAT	TCTGAGCAAC	CTGATTTCTC	TGGACACAAA	1000
TATGGCCCTT	TCGGCCCTGA	GGAGAGTAGT	TATGGCTCAC	1040
CTTTTACTCC	GGCTAAGAGA	CCTAAGAGGA	AAGTTGCCCC	1080
TAAGAGGAGA	CAGGAAAGAC	CAGTTGCTCC	TCCAAAGAAA	1120
AGAAGAAGAA	AAATACATAG	GATGGATCAT	TATGCTGCGG	1160
AAACTCGTCA	GGACAAAATG	ACAAATCCTC	TGAGGGAAAT	1200
CGACAAAATT	GTGGGGCAAT	TAATGGATGG	ACTGAAACAA	1240
CTAAAACTGC	GTCGGTGTGT	CAACGTCATC	TTTGTGCGAG	1280
ACCATGGAAT	GGAAGATGTC	ACATGTGATA	GAAGTGAGTT	1320
CTTGAGTAAT	TACCTAATA	ATGTGGATGA	TATTACTTTA	1360
GTGCCTGGAA	CTCTAGGAAG	AATTCGATCC	AAATTTAGCA	1400
ACAATGCTAA	ATATGACCCC	AAAGCCATTA	TTGCCAATCT	1440
CACGTGTAAA	AAACCAGATC	AGCACTTTAA	GCCTTACTTG	1480
AAACAGCACC	TTCCCAAACG	TTTGCACTAT	GCCAACAACA	1520
GAAGAATTGA	GGATATCCAT	TTATTGGTGG	AACGCAGATG	1560
GCATGTTGCA	AGGAAACCTT	TGGATGTTTA	TAAGAAACCA	1600
TCAGGAAAAT	GCTTTTTTCCA	GGGAGACCAC	GGATTTGATA	1640
ACAAGGTCAA	CAGCATGCAG	ACTGTTTTTTG	TAGGTTATGG	1680
CCCAACATTT	AAGTACAAGA	CTAAAGTGCC	TCCATTTGAA	1720
AACATTGAAC	TTTACAATGT	TATGTGTGAT	CTCCTGGGAT	1760
TGAAGCCAGC	TCCTAATAAT	GGGACCCATG	GAAGTTTGAA	1800
TCATCTCCTG	CGCACTAATA	CCTTCAGGCC	AACCATGCCA	1840
GAGGAAGTTA	CCAGACCCAA	TTATCCAGGG	ATTATGTACC	1880
TTCAGTCTGA	TTTTGACCTG	GGCTGCACTT	GTGATGATAA	1920
GGTAGAGCCA	AAGAACAAGT	TGGATGAACT	CAACAAACGG	1960
CTTCATACAA	AAGGGTCTAC	AGAAGAGAGA	CACCTCCTCT	2000

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ATGGGCGACC	TGCAGTGCTT	TATCGGACTA	GATATGATAT	2040
CTTATATCAC	ACTGACTTTG	AAAGTGGTTA	TAGTGAAATA	2080
TTCCTAATGC	TACTCTGGAC	ATCATATACT	GTTTCCAAAC	2120
AGGCTGAGGT	TTCCAGCGTT	CCTGACCATC	TGACCAGTTG	2160
CGTCCGGCCT	GATGTCCGTG	TTTCTCCGAG	TTTCAGTCAG	2200
AACTGTTTGG	CCTACAAAAA	TGATAAGCAG	ATGTCCTACG	2240
GATTTCCTCTT	TCCTCCTTAT	CTGAGCTCTT	CACCAGAGGC	2280
TAAATATGAT	GCATTCCCTG	TAACCAATAT	GGTTCCAATG	2320
TATCCTGCTT	TCAAACGGGT	CTGGAATTAT	TTCCAAAGGG	2360
TATTGGTGAA	GAAATATGCT	TCGGAAAGAA	ATGGAGTTAA	2400
CGTGATAAGT	GGACCAATCT	TCGACTATGA	CTATGATGGC	2440
TTACATGACA	CAGAAGACAA	AATAAAACAG	TACGTGGAAG	2480
GCAGTTCCAT	TCCTGTTCCA	ACTCACTACT	ACAGCATCAT	2520
CACCAGCTGT	CTGGATTTCA	CTCAGCCTGC	CGACAAGTGT	2560
GACGGCCCTC	TCTCTGTGTC	CTCCTTCATC	CTGCCTCACC	2600
GGCCTGACAA	CGAGGAGAGC	TGCAATAGCT	CAGAGGACGA	2640
ATCAAAATGG	GTAAGAAGAC	TCATGAAGAT	GCACACAGCT	2680
AGGGTGCGTG	ACATTGAACA	TCTCACCAGC	CTGGACTTCT	2720
TCCGAAAGAC	CAGCCGCAGC	TACCCAGAAA	TCCTGACACT	2760
CAAGACATAC	CTGCATACAT	ATGAGAGCGA	GATTTAACTT	2800
TCTGAGCATC	TGCAGTACAG	TCTTATCAAC	TGGTTGTATA	2840
TTTTTTATATT	GTTTTTGTAT	TTATTAATTT	GAAACCAGGA	2880
CATTA AAAAT	GTTAGTATTT	TAATCCTGTA	CCAAATCTGA	2920
CATATTATGC	CTGAATGACT	CCACTGTTTT	TCTCTAATGC	2960
TTGATTTAGG	TAGCCTTG TG	TTCTGAGTAG	AGCTTGTAAT	3000
AAATACTGCA	GCTTGAGAAA	AAGTGGAAGC	TTCTAAATGG	3040
TGCTGCAGAT	TTGATATTTG	CATTGAGGAA	ATATTAATTT	3080
TCCAATGCAC	AGTTGCCACA	TTTAGTCCTG	TACTGTATGG	3120
AAACACTGAT	TTTGTAAGT	TGCCTTTATT	TGCTGTTAAC	3160
TGTTAACTAT	GACAGATATA	TTTAAGCCTT	ATAAACCAAT	3200
CTTAAACATA	ATAAATCACA	CATTCAAGTTT	TAAAAA AAAA	3240
AAAAAAAAAA	A			3251

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 915
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(ix) FEATURE:

- (A) NAME/KEY: A2058 ATX protein
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Met	Ala	Arg	Arg	Ser	Ser	Phe	Gln	Ser	Cys	Gln	Ile		
1				5					10				
Ile	Ser	Leu	Phe	Thr	Phe	Ala	Val	Gly	Val	Ser	Ile		
		15					20						
Cys	Leu	Gly	Phe	Thr	Ala	His	Arg	Ile	Lys	Arg	Ala		
25					30					35			
Glu	Gly	Trp	Glu	Glu	Gly	Pro	Pro	Thr	Val	Leu	Ser		
			40					45					
Asp	Ser	Pro	Trp	Thr	Asn	Ile	Ser	Gly	Ser	Cys	Lys		
		50			55						60		
Gly	Arg	Cys	Phe	Glu	Leu	Gln	Glu	Ala	Gly	Pro	Pro		
				65					70				
Asp	Cys	Arg	Cys	Asp	Asn	Leu	Cys	Lys	Ser	Tyr	Thr		
		75					80						
Ser	Cys	Cys	His	Asp	Phe	Asp	Glu	Leu	Cys	Leu	Lys		
85				90						95			
Thr	Ala	Arg	Gly	Trp	Glu	Cys	Thr	Lys	Asp	Arg	Cys		
			100					105					
Gly	Glu	Val	Arg	Asn	Glu	Glu	Asn	Ala	Cys	His	Cys		
						115					120		
Ser	Glu	Asp	Cys	Leu	Ala	Arg	Gly	Asp	Cys	Cys	Thr		
				125					130				
Asn	Tyr	Gln	Val	Val	Cys	Lys	Gly	Glu	Ser	His	Trp		
		135					140						
Val	Asp	Asp	Asp	Cys	Glu	Glu	Ile	Lys	Ala	Ala	Glu		
145					150					155			
Cys	Pro	Ala	Gly	Phe	Val	Arg	Pro	Pro	Leu	Ile	Ile		
			160					165					
Phe	Ser	Val	Asp	Gly	Phe	Arg	Ala	Ser	Tyr	Met	Lys		
		170				175					180		
Lys	Gly	Ser	Lys	Val	Met	Pro	Asn	Ile	Glu	Lys	Leu		
				185					190				
Arg	Ser	Cys	Gly	Thr	His	Ser	Pro	Tyr	Met	Arg	Pro		
		195					200						
Val	Tyr	Pro	Thr	Lys	Thr	Phe	Pro	Asn	Leu	Tyr	Thr		
205					210					215			
Leu	Ala	Thr	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile		
			220					225					
Val	Gly	Asn	Ser	Met	Tyr	Asp	Pro	Val	Phe	Asp	Ala		
		230				235					240		
Thr	Phe	His	Leu	Arg	Gly	Arg	Glu	Lys	Phe	Asn	His		
				245					250				
Arg	Trp	Trp	Gly	Gly	Gln	Pro	Leu	Trp	Ile	Thr	Ala		
		255					260						
Thr	Lys	Gln	Gly	Val	Lys	Ala	Gly	Thr	Phe	Phe	Trp		
265					270					275			
Ser	Val	Val	Ile	Pro	His	Glu	Arg	Arg	Ile	Leu	Thr		
			280					285					
Ile	Leu	Arg	Trp	Leu	Thr	Leu	Pro	Asp	His	Glu	Arg		
		290				295					300		
Pro	Ser	Val	Tyr	Ala	Phe	Tyr	Ser	Glu	Gln	Pro	Asp		
				305					310				

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Phe	Ser	Gly	His	Lys	Tyr	Gly	Pro	Phe	Gly	Pro	Glu
		315					320				
Glu	Ser	Ser	Tyr	Gly	Ser	Pro	Phe	Thr	Pro	Ala	Lys
325					330					335	
Arg	Pro	Lys	Arg	Lys	Val	Ala	Pro	Lys	Arg	Arg	Gln
		340						345			
Glu	Arg	Pro	Val	Ala	Pro	Pro	Lys	Lys	Arg	Arg	Arg
350						355					360
Lys	Ile	His	Arg	Met	Asp	His	Tyr	Ala	Ala	Glu	Thr
			365						370		
Arg	Gln	Asp	Lys	Met	Thr	Asn	Pro	Leu	Arg	Glu	Ile
		375				380					
Asp	Lys	Ile	Val	Gly	Gln	Leu	Met	Asp	Gly	Leu	Lys
385					390					395	
Gln	Leu	Lys	Leu	Arg	Arg	Cys	Val	Asn	Val	Ile	Phe
			400					405			
Val	Gly	Asp	His	Gly	Met	Glu	Asp	Val	Thr	Cys	Asp
410						415					420
Arg	Thr	Glu	Phe	Leu	Ser	Asn	Tyr	Leu	Thr	Asn	Val
			425						430		
Asp	Asp	Ile	Thr	Leu	Val	Pro	Gly	Thr	Leu	Gly	Arg
		435				440					
Ile	Arg	Ser	Lys	Phe	Ser	Asn	Asn	Ala	Lys	Tyr	Asp
445					450					455	
Pro	Lys	Ala	Ile	Ile	Ala	Asn	Leu	Thr	Cys	Lys	Lys
			460					465			
Pro	Asp	Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	Gln	His
		470				475					480
Leu	Pro	Lys	Arg	Leu	His	Tyr	Ala	Asn	Asn	Arg	Arg
			485						490		
Ile	Glu	Asp	Ile	His	Leu	Leu	Val	Glu	Arg	Arg	Trp
		495					500				
His	Val	Ala	Arg	Lys	Pro	Leu	Asp	Val	Tyr	Lys	Lys
505					510					515	
Pro	Ser	Gly	Lys	Cys	Phe	Phe	Gln	Gly	Asp	His	Gly
			520					525			
Phe	Asp	Asn	Lys	Val	Asn	Ser	Met	Gln	Thr	Val	Phe
530						535					540
Val	Gly	Tyr	Gly	Pro	Thr	Phe	Lys	Tyr	Lys	Thr	Lys
			545						550		
Val	Pro	Pro	Phe	Glu	Asn	Ile	Glu	Leu	Tyr	Asn	Val
		555					560				
Met	Cys	Asp	Leu	Leu	Gly	Leu	Lys	Pro	Ala	Pro	Asn
565					570					575	
Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Arg
			580					585			
Thr	Asn	Thr	Phe	Arg	Pro	Thr	Met	Pro	Glu	Glu	Val
	590					595					600
Thr	Arg	Pro	Asn	Tyr	Pro	Gly	Ile	Met	Tyr	Leu	Gln
			605						610		
Ser	Asp	Phe	Asp	Leu	Gly	Cys	Thr	Cys	Asp	Asp	Lys
		615					620				
Val	Glu	Pro	Lys	Asn	Lys	Leu	Asp	Glu	Leu	Asn	Lys
625					630					635	

